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Image Analysis Techniques for the Study of Fish School Structure from Acoustic Survey Data

D.G. Reid and E.J. Simmonds

Scottish Office Agriculture and Fisheries Department, Marine Laboratory, Victoria Road, Torry, P.O. Box 101, Aberdeen, AB9 8DB, U.K.

Reid, D.G., and E.J. Simmonds. 1993. Image analysis techniques for the study of fish school structure from acoustic survey data. Can. J. Fish. Aquat. Sci. 50: 886–893.

A new image analysis based processing system is described for acoustic fisheries data. In a series of a simple steps the system can identify schools and display their positions on a screen. Modifications to the identification process can be entered interactively by the operator. The system provides a wide range of parameters associated with each school, e.g., height, width, depth, shape, density, that can be entered into a database. Applications of this type of system for improving the accuracy of acoustic stock assessment, studying fish distribution patterns, and elucidating fish schooling behaviour are discussed.

On décrit un nouveau système fondé sur l'analyse d'image pour le traitement des données acoustiques sur la pêche. Par une série d'étapes simples, le système peut reconnaître les bancs de poissons et afficher leur position sur un écran. Le préposé au système peut apporter, de manière interactive, des modifications au processus d'identification. Le système fournit une gamme étendue de paramètres pour chaque banc, p.ex., hauteur, largeur, forme, densité, qui peuvent être consignées dans une banque de données. On discute des applications de ce type de système pour accroître la précision de l'évaluation des stocks par méthode acoustique, pour étudier les modes de distribution des poissons et pour comprendre le comportement de rassemblement en bancs de poissons.

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Acoustic methods have been used to determine abundance and distribution of pelagic fish stocks for over 20 yr. In marine fisheries, this has generally been achieved by echo-integration (Forbes and Nakken 1972) combined with size and species composition data from associated trawling (Nakken and Dommasnes 1975). This approach has the obvious disadvantage that it is unable to reveal any more than the number or biomass of fish from groups of transmissions. In some cases, it is possible to divide the water column into different layers and integrate separately for each layer (e.g., 5–50, 50–200 m). This approach gives a slightly more detailed picture of the distribution of the fish in the water column, but, in practice, it is restricted to a small number of integration layers, and integrated volumes have little relation to individual fish schools.

Custom-built echo-sounders that can collect a large number of independent samples over depth from each transmission have been available for some time. This type of echo-sounder system is now available commercially. It should, therefore, be possible to use these data to identify and classify the fish schools in which we are interested, automatically and precisely. This would result in two major improvements in the value of acoustic surveys. First, it will be possible to include only the echo energy from actual schools of fish in the abundance estimate. This would avoid the inclusion of other scatterers that are in the water column, most importantly, plankton. To date, this type of sorting has largely been carried out manually. Second, it will be possible to study the structure of the fish population in much more detail than has previously been possible (e.g., the size, shape, density, position, and depth of each school). This latter approach has been used by Azzali et al. (1981, 1985) on sardine schools in the Adriatic.

The main difficulty for the researcher wishing to analyse fish schools in any detail lies in the degree of data processing required. Azzali et al. (1981) manually determined the height, width, and volume for 500 schools of Adriatic sardine. However, it would be impossible to process the amount of data available from a normal acoustic survey without some form of graphical or automatic data processing. To illustrate this, with one transmission every second, samples collected every 0.5 m, and in an average depth of 100 m, a 1-h survey would produce 720 000 data points! The most advanced computer-assisted processing systems, such as the BI500 (Knudsen 1991) from Simrad, still require manual extraction of information on the structure of fish schools.

Image analysis object shape criteria have previously been used in the analysis of acoustic survey data by Souid (1987) and Masse and Rouxel (1991). However, no details of the method of school acquisition or its speed or degree of automation were provided, and no use was made of object recognition algorithms. In this paper, we describe the application of a wide range of image processing methods to the analysis of acoustic data. The technique allows the automatic identification of fish schools and the determination of size, shape, volume, density, position, depth, height above the seabed, and a number of other parameters for each individual school.

Materials and Methods

Data Collection

A dual-frequency Simrad EK400 echo-sounder system provides two calibrated analogue outputs for data recording. Two frequencies from a choice of 38, 120, and 200 kHz are

selected for any one survey. Two methods of obtaining dual-frequency data have been used: the single-controller standard system and the dual-controller with a common clock, the second providing twice the data rate. The sounders were operated with a 1-ms pulse length and a 3-kHz receiver bandwidth. In both cases the two transceivers are triggered sequentially and the calibrated outputs are gated, detected, and digitised to 12-bit precision at 0.5-m (0.666-ms) intervals. With a 1-ms pulse length, independent samples are spaced 1 ms apart, independent of receiver bandwidth. The sampling rate of 0.666 ms is insufficient to provide a full reconstruction of the received echoes. However, reconstruction is not required for evaluating shoal shape. The subsequent processing steps we describe here will reduce the high-frequency detail in the signal. This detail is, basically, a property of random-phase relationships between echoes from multiple targets within the same pulse volume and is not useful for determination of school shape or evaluation of school echo energy. The detector and ADC system provide voltage linearity of better than ±1% from full scale of 10 V p-p down to 30 mV p-p (50 dB) and ±4% to 3 mV p-p. The digitised system is controlled by a Computer Automation 4/90 computer. Samples are collected from the surface to 20 m below the seabed (Simmonds and MacLennan 1990) to a maximum of 1024 samples per transmission. The data are collected into 8-kilobyte blocks along with header information on vessel position, time, and date obtained by serial link from the ship's navigation system or a portable navigator. The data blocks are recorded on 150-megabyte DC600 cartridges. The block rate and thus the time and position recording rate is approximately 4 per minute.

Image Processing Hardware

Image processing was carried out using an Innovision image analysis system. The main processor is a Heurikon 86030 running under Unix. The computer is equipped with a number of purpose-built, high-speed image processing boards, supplied by Imaging Technology Incorporated, USA. These are full-width VME boards mounted in a VME rack along with the main processor and memory expansion. The individual boards necessary to implement this type of processing and analysis were an analogue to digital input/output board (ADI150); a frame store (FB150), allowing storage of up to four 512 × 512 × 8 bit images; an arithmetic logic unit (ALU150), for most calculations; a histogram-frequency analysis board (FB150), allowing rapid histogram equalization; and a specific board for rapid implementation of convolution filters (see below) (RTMP150 with a CM-150-RTS module). Operating software is provided by Imaging Technology Inc. as "C" callable functions, which have been incorporated into a custom built "C" (Greenhills C) program written by the authors. Further details of the program's operation are given below.

Data Entry

Each data point, representing the echo amplitude (voltage) from 0.5 m of the water column, from a single transmission, is produced by the digitiser as a 12-bit number. This must first be reduced to the eight bits required by the image processing hardware. The choice of which bits to remove will depend on the particular application. Removing the least significant bits will tend to reduce the noise in the recording, but will also remove much of the acoustic return from weak scatterers, such as plankton. Clipping the most significant bits will tend to result in a loss of dynamic range, and all strong scatterers will have a value of 255. In this particular design, we removed the least significant two bits and clipped the resulting 10-bit number to a maximum of 255. The reduction to eight bits is only necessary for the image processing and should be allowed for in the final calculations of fish biomass. If required, the original 12-bit data can be used subsequently.

The data points are then built into a series of image files. Each file is constructed as a 128 × 512 matrix. The columns represent 128 consecutive transmissions, and the rows represent the voltages from consecutive 0.5-m samples through the water column. The subsequent image file, loaded into the frame store (an area in the machine's memory for image storage and manipulation) and presented on a colour visual display unit (VDU), fills a quarter of a 512 × 512 pixel screen. Four consecutive images result in a completed screen, 512 transmissions wide, which, using a suitable pseudocolour look-up table, is analogous to the display on a colour sounder (Fig. 1a). A look-up table is a software mechanism to give a different colour to each of the full range of possible point values (in this case, 0–255). Each pixel (or point) on the screen (and in the frame buffer) represents a single sample from a single transmission. Each quarter screen image file also includes header information on time, date, position, and type of sounder.

Image Processing

Once loaded into the frame store the echogram image can be processed to allow extraction of the school parameters. The original image containing the raw data is retained for biomass estimation. Processing for school identification is carried out using the three steps described below.

The central mechanism used in much of the image processing described below is the convolution kernel. Simply described, convolution involves the averaging of a single point (pixel) with its eight nearest neighbours. At its simplest, we would add the values of the nine pixels together and take an average, which would then be assigned to that pixel. The convolution kernel is used to apply a weighting factor to this local averaging process; for instance, a kernel of the following design:

$$K_{ij} = \begin{vmatrix} 1 & 1 & 1 \\ 1 & 4 & 1 \\ 1 & 1 & 1 \end{vmatrix}.$$

This would average the result for four times the value of the central pixel plus the values of its neighbours. Different weighting factors, i.e., different kernels, will produce a range of results. For further information on convolution filters, see Castleman (1979) and Gonzalez and Wintz (1987).

Smoothing

The first step is to smooth the image (Fig. 1b) to remove unwanted noise and prepare the image for further analysis. Smoothing reduces the local (pixel-to-pixel) variability in the image and has two important effects. First, it reduces the variability within objects, such as schools or the seabed. A high degree of local variability in objects would make it difficult to carry out a reliable binary thresholding (see below). In general, the images of fish schools differ from that of the seabed, in the high degree of variance in values (echo amplitude) on a pixel-to-pixel basis. For a uniform fish density with several fish per pulse volume the echo amplitude probability density function (PDF) theoretically follows a Rayleigh distribution. Provided

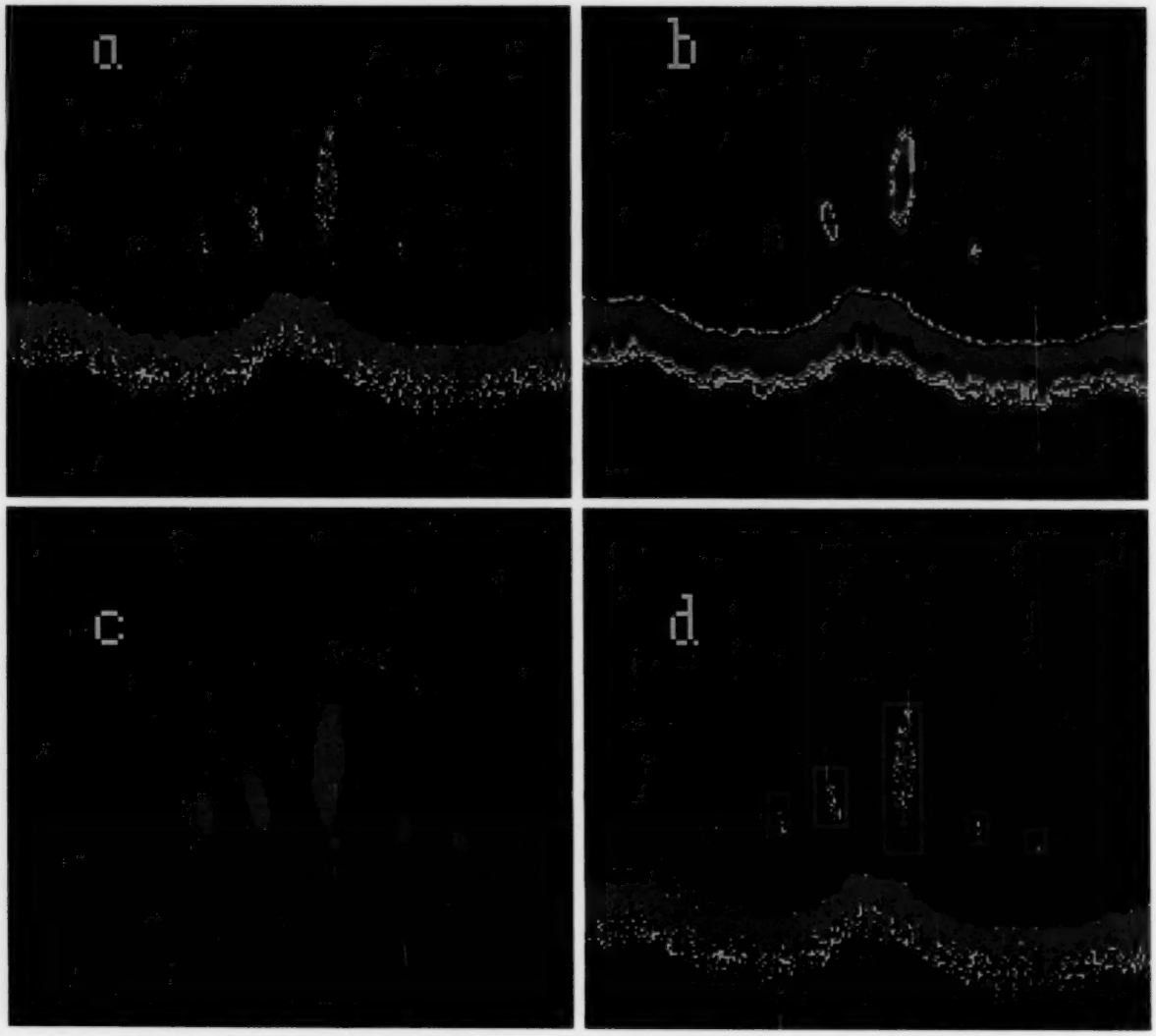

FIG. 1. Example analysis of an echogram displaying the seabed and five herring schools. (a) Original image; (b) "blurred" image; (c) binary image of schools; (d) final image, showing detected schools marked with boxes.

the fish locations are randomly located on the scale of a wavelength (3.9 cm) the individual samples making up a school should be uncorrelated. High-amplitude pixels may be adjacent to relatively low-amplitude pixels. The seabed tends to have a more uniformly high value, although this may also be due to receiver saturation. After smoothing, the seabed will, therefore, tend to retain an overall high pixel value, while the schools will average out to a generally lower value. This makes it simpler to determine a threshold value for transformation to a binary image (see below). A second effect of smoothing is that it reduces the amplitude of background reverberation, caused by plankton or other isolated targets. Smoothing is carried out using pixel averaging. A 3×3 "blur" filter with the following kernel is used:

$$K_{ij} = \begin{vmatrix} 1 & 2 & 1 \\ 2 & 1 & 2 \\ 1 & 2 & 1 \end{vmatrix} .$$

The value of each pixel of index (x,y) is then given by the following convolution:

$$(1) \quad R(x,y) = \frac{1}{C} \sum_{ij} K_{ij} P(x + i, y + j) \text{ for } i, j = -1, 0, 1$$

where $R(x,y)$ is the resultant pixel value, K_{ij} is the kernel element, $P(x,y)$ is the original pixel value, and C is a constant that can be given by

$$(2) \quad C = \sum_{ij} K_{ij} .$$

The constant is applied to give an average of the summed pixel values and to give a resultant pixel value in the same range as the original.

An alternative method of smoothing would be to use a low-pass filter. This uses a slightly different kernel with a greater emphasis on the central element:

$$K_{ij} = \begin{vmatrix} 1 & 1 & 2 \\ 2 & 4 & 2 \\ 1 & 2 & 1 \end{vmatrix}.$$

Both these filters tend to smooth the image. The main difference in the output image in this case is that the low-pass filter tends to result in the pixel values having a slightly greater dynamic range and, in addition, the transition from object to background is smoother. Therefore, the choice of a value for binary thresholding may be more difficult. For this reason, we chose to use the "blur" rather than the low-pass filter.

Larger filter matrices or kernels (e.g., 5 × 5) were found to be less useful than the 3 × 3 designs. All smoothing convolution filters tend to spread objects out, which makes them appear larger in the processed image than they actually are, and also to merge closely adjacent objects (e.g. schools and/or the seabed). This tendency is increased with larger filter matrices. For further information on convolution filters, see Castleman (1979) and Gonzalez and Wintz (1987).

After smoothing, it is possible to further process the image using a "sharpen" filter. This type of filter is designed to highlight edges and so appears to sharpen the image, or make it apparently clearer. It also functions to counteract the spreading effect of the smoothing filter by removing the outermost layers of pixels from a blurred object, which tend to be of low intensity. The following kernel was used:

$$K_{ij} = \begin{vmatrix} -1 & -1 & -1 \\ -1 & 9 & -1 \\ -1 & -1 & -1 \end{vmatrix}.$$

The value of each pixel of coordinates (x,y) is then calculated using equation (1) with $C = 1$.

We did not use such a filter, as we found that its use did not appreciably enhance the final result. Proper selection of a threshold value (see below) was sufficient to counteract the spreading effect of the smoothing filter.

Binary thresholding for bottom detection

The next step is to identify the seabed in the image, determine its position in each transmission, and then remove it from the image prior to defining the fish schools. The last part is necessary because without deletion, the seabed will be seen as another object, no different to the object recognition algorithm than any schools that may also be present. The image is first transformed to a binary configuration (Fig. 1c). This is done by thresholding the image. All the pixels (representing individual samples) have a value in the range of 0–255. To threshold the image, a threshold value is chosen between 0 and 255. Any pixel (sample) whose value is below this threshold (the background) is set to 0, and any pixel whose value is higher (the object) is set to 1. This can be expressed by the equation

$$(3) \quad B(x,y) = (P(x,y) \geq T)$$

where $B(x,y)$ is the resultant binary pixel value, either 0 or 1, $P(x,y)$ is the original pixel value, between 0 and 255, and T is the chosen threshold value, between 0 and 255. The right-hand side of the equation is a logical expression giving a value of 1 when true and 0 when false.

The value chosen for such a threshold is clearly critical and will depend to a high degree on the particular data set and preceeding smoothing operations. A frequency histogram of the pixel values can be used to assist in setting an effective threshold. For further information on histogram analysis, see Castleman (1979) and Gonzalez and Wintz (1987). The threshold can also be set to a particular value. In this case, we chose a threshold of 100, as we had found from experience that this value allowed retention of the seabed but eliminated all but the very densest schools.

This first thresholding is to allow identification of the seabed. A high threshold is used, as the seabed is normally the strongest object in the image, i.e., it contains the pixels with the highest values. Complications may arise, however, if the resultant bottom image is split into a number of parts or if there are other objects, such as very dense schools, that remain after thresholding. To control for this the binary image is first eroded through two cycles. In simple terms, for erosion of a binary image, a layer of pixels is removed from any object in the image. Binary erosion is carried out according to the following algorithm:

$$\begin{vmatrix} P(x-1, y+1) & P(x,y+1) & P(x+1, y+1) \\ P(x-1, y) & P(x,y) & P(x+1, y) \\ P(x-1, y-1) & P(x,y-1) & P(x+1, y-1) \end{vmatrix}$$

representing the pixel $P(x,y)$ and its eight nearest neighbours. The resulting value of pixel $P(x,y)$ is given by

$$(4) \quad B(x,y) = \min[P(x+i, y+j)] \quad \text{for } i = -1, 0, 1; j = -1, 0, 1$$

where $B(x,y)$ is the resultant binary pixel value, $P(x,y)$ is the original binary pixel value, and i, j represent the vertical and horizontal displacement, respectively, from the central pixel.

Thus, if a central pixel has one or more zero (background) pixels among its eight neighbours, it is set to 0, regardless of its original value. Thus, isolated or small groups of nonzero pixels are removed from the image. In this case the image is eroded twice. Smaller objects, or those containing embedded zero pixels, such as fish schools, are likely to be reduced or eliminated.

The image is then dilated by three cycles. Dilation is the opposite of erosion, according to the equation

$$(5) \quad B(x,y) = \max[P(x+i, y+j)] \quad \text{for } i = -1, 0, 1; j = -1, 0, 1$$

where $B(x,y)$ is the resultant binary pixel value, $P(x,y)$ is the original binary pixel value, and i, j represent the vertical and horizontal displacement, respectively, from the central pixel.

Thus, if a central pixel has one or more nonzero (object) pixels among its eight neighbours, it is set to 1, regardless of its original value. Thus, any remaining groups of nonzero pixels are magnified and should result in their becoming slightly larger than they were prior to the erosion steps.

In simple terms the two steps of erosion followed by dilation will eliminate any small objects and then restore those remaining to approximately their original dimensions. The third dilation cycle is to ensure that the final image of the seabed includes all the possible seabed pixels, i.e., the object (the seabed) is made slightly larger than the actual seabed, and most importantly, an extra layer of pixels is added above it. If there is still more than one object in the image, the remaining objects are then either joined together to form a continuous seabed or rejected as not part of the seabed.

Once a single, continuous bottom has been found, the depth of the seabed in each column (transmission) is recorded, and the seabed and everything below it is erased from the original image. The remaining nonzero pixels should now represent only the observed fish schools. The data on the located seabed depth are added to the database and used to determine the depth and gradient under a school.

If the thresholding to find the seabed is carried out carefully, it is possible to identify and quantify fish schools close to or even in contact with the bottom. This is not possible when the lower periphery of such schools is close in pixel value to those making up the seabed. Such cases also create problems for bottom-following algorithms in echo-sounders. In the case of schools actually in contact with the seabed, it is essential to discard the samples in the band corresponding to the half pulse length immediately above the seabed. On sloping or rough bottoms, a wider band may have to be excluded.

Identification of schools

The final step is to identify the fish schools left in the image after smoothing and deletion of the seabed. The blurred image, minus seabed, is thresholded again to produce a binary picture of the remaining objects (schools). A much lower threshold is used (in this case, 5), as schools are usually much less intense than the seabed. The frequency histogram can again be used to help determine a suitable threshold value. The subsequent binary image is then eroded, as above, by two cycles and dilated by two. This has the dual functions of removing very small objects and smoothing the outlines of larger ones. The image is then passed through a series of object-labelling routines supplied by the manufacturer of the image processing hardware. Briefly, these assign each object pixel to a larger object based on "eight connectivity." Every nonzero (object) pixel has eight neighbours, and if one or more of those are also object pixels, then they become part of a mask that describes the object. In this way the system assigns all the object pixels to masks whose locations and dimensions are known.

So far the dimensions of the school are only known in terms of x and y coordinates on the screen. The next step is to convert these to the true dimensions of the original school. Each pixel represents a single sample from a single transmission, and samples are taken at 0.5-m (0.666-s) intervals. Vertical dimensions (in metres) are calculated from the height, in pixels, multiplied by the sample size.

The measured horizontal dimensions of a pixel are determined from the ship's speed and the transmission speed. The transmission speed is dependent on the depth setting of the echo-sounder such that the deeper the sounder is set, the longer the interval between transmissions. In this case the survey was conducted with the sounder set at 150 m, equivalent to one transmission every 0.7 s. The measured pixel width is found by multiplying the ship speed (metres per second) by the transmission interval (seconds). The horizontal dimensions (in metres) are then calculated using the width of a pixel (in metres), multiplied by the number of pixels across the object. Both these calculated dimensions also have to be corrected for artifacts introduced by, respectively, pulse length and beam width.

The height of the school image on the echo-sounder is actually a combination of the true height of the school plus the height of the independent sample. The first part of the school appearing on the echo-sounder is from the leading edge of the pulse encountering the upper edge of the school. The echo will continue until the trailing edge of the pulse passes beyond the lower edge of the school. The time taken for the whole echo to arrive is, thus, increased by the time of one pulse. The distance travelled by the pulse in that time is, effectively, half the pulse length, as range is calculated from the equation

$$(6)\quad D = \frac{(T \cdot C)}{2}$$

where D = range (metres), T = time taken (seconds), and C = speed of sound in water (metres per second).

The measured height is then corrected for pulse length with the equation

$$(7)\quad Y_{true} = Y_{meas} - \frac{\tau}{2}$$

where Y_{true} = actual height of the school, Y_{meas} = measured height of the school, and τ = pulse length.

As in the case of the school height, the school width must also be corrected, in this case for artifacts due to the beam width. The echo-sounder beam can be considered as a cone. As the ship moves forward, any school will first be encountered by the leading edge of the beam. The true width of the school would be the distance travelled until the whole school had passed through this leading edge. However, the school will continue to produce an echo until it leaves the trailing edge of the beam. Hence, the width of the school on the echogram is the true width plus the beam width. A further complication is that the beam width increases with depth, and so the correction for beam width is dependent on the depth of the school.

The true horizontal dimensions of the school can then be determined by the equation (after Smith 1970)

$$(8)\quad X_{true} = X_{meas} - 2\tan\Theta\left[\frac{(D_{max} + D_{min})}{2}\right]$$

where X_{true} = actual width of the school, Y_{meas} = measured width of the school, Θ = half angle of the beam, D_{max} = range to the far side of the school, and D_{min} = range to the near side of the school.

There is one further consideration regarding school width, and to a lesser extent, height. The image of the school on the echogram can be considered as a two-dimensional slice. There is no guarantee that this slice has been taken through the middle of the school; indeed, it is most likely that it will be off-centre. Over a large number of schools sampled, it is possible to assume that they will have been encountered at random and that a full range of slices (both off-centre and through the centre) will have been taken. The apparent widths for any particular school will, thus, range from the actual maximum width through to close to 0. It is possible to correct for this using the equation

$$(9)\quad W_{true} = \frac{4W_{meas}}{\Pi}$$

where W_{true} = actual school diameter and W_{meas} = observed school diameter.

This relationship is only strictly valid when the cross section of the school is circular. Evidence from tank experiments suggests that, at least in the case of Atlantic herring (*Clupea harenqus*), the species we are currently studying, this is probably true (Pitcher and Partridge 1979). It is less simple to correct for height here, as the school cannot be considered as a sphere. We have assumed the shape of herring schools to be symmetrical, and therefore, a single slice will convey information on

TABLE 1. Example of the type of output produced by the system. It should be noted that in this case no correction for beam width or pulse length has been carried out.

	School No.				
	1	2	3	4	5
School area (m^2)	74	173	598	54	32
Mean intensity	652	1645	8815	1743	1502
Total energy	26729	157950	2926640	52923	27028
Depth (m)	148	148	140	143	146
Height off bottom (m)	15.5	18	19.5	15	16
Shoal depth (m)	132.5	130	120.5	127.5	130.0
X size (m)	21.6	36.0	43.2	21.6	21.6
Y size (m)	6.5	9.5	25.0	5.0	3.5
X:*Y* ratio	3.3	3.8	1.7	4.3	6.2
Centre of geometry[a]					
dX	0	0	+0.17	+0.17	0
dY	+0.8	0	+0.1	+0.1	0
Centre of mass[a]					
dX	0	+0.1	+0.08	0	0
dY	−0.08	0	+0.16	−0.1	−0.14

[a]Centres of geometry and mass are calculated to the nearest pixel. The *X* and *Y* displacements of these values from the geometric centre of the bounding rectangle (see Fig. 1d) are then determined. These data are then presented as proportions of the *X* and *Y* dimensions of the bounding rectangle.

school shape. Correction for true height would require further information on the actual shape of schools.

The information on school position and dimensions is then used to refer back to the original image in order to find the value of each of the object (school) pixels. All the steps in the analysis described are on data clipped to 8 bits (see above). To derive the acoustic intensity of the echo from the whole school, we take the original 12-bit values which are then squared and summed. Other information such as depth, time, and position can also be extracted from the image file header. As the depth of the seabed at each transmission has been found and stored (see above), it is also possible to determine the height of the school off the bottom and, in addition, the slope of the bottom immediately under the school. Each school is then identified on the display screen by a box superimposed on the original image (Fig. 1d). At this point the operator can choose to accept the computer's identification of the school. If so, the parameters determined for the school are entered into the database; if not, they are discarded. The operator can also choose to identify objects that the algorithm has failed to find, and these can also be entered into the database. These will usually be very small or very low amplitude schools. It is important to realise that all the image processing steps outlined are only intended to delineate the schools as objects. The final information on the energy returned from the school must be taken from the original, untreated image.

Sample Calculation

The system is very rapid. With transmissions every 0.7 s, a full screen, 512 pixels wide, would take approximately 6 min to collect. The analytical steps outlined above would take approximately 10 s to complete, depending on the number of objects in the image. The system is, therefore, clearly capable of real-time applications in fisheries acoustics, as well as in subsequent analysis. The approximate timing for each step in a typical sequence is as follows: load a full screen from disk = 8 s, blur image = <0.5 s, threshold for seabed detection = <0.5 s, erode (×2) and dilate (×3) image = 1 s, erase seabed from image = <0.5 s, identify and label objects = 5 s, and calculate parameters = 3 s. The last two steps will vary in length according to the number of objects found. In this example, 20 schools were identified in the full-width image. Seabed detection may also be slower when the image is broken up or contains a number of very strong school echoes.

An example of the program output for the schools identified in Fig. 1 is given in Table 1. The values given are the cross-sectional area of the school; the mean echo intensity; the total echo energy recorded for the school, from which the fish biomass can be calculated; the water depth; the water depth of the centre of the school; horizontal and vertical size, and the ratio of these; and the displacements of the centres of mass and geometry of each school from the centre of the bounding rectangle. These last values, together with the ratio of height to width, are possible indicators of school shape. Other, more complex shape parameters could be determined that would allow comparison between school shapes (e.g., those described in Souid (1987), Flusser (1992), and Sardana et al. (1992)). Seabed gradient and bottom features under the schools could also be included.

The mechanism for identifying schools described here contains a number of processing steps. These (especially smoothing and thresholding) may result in the shape of the identified school being subtly different from that of the real school. For example, the outline may be smoother. The possibility exists, therefore, that this may introduce a small degree of bias in the parameters determined for the schools. It is reasonable to assume that any bias will be similar for all schools of a particular species, and for comparison purposes the bias can probably be ignored. Experience with the system has shown that there is no significant bias in the determination of fish biomass for the identified schools.

Discussion

The system described above has a number of advantages for the scientist working in fisheries acoustics. First, it should allow

a more accurate quantification of the fish surveyed. Through a series of image processing steps the computer is able to identify most, if not all, of the potential fish schools in a given echogram and present them to the operator. The operator can then accept or modify the computer's interpretation of the echogram. In previous methods (Dalen and Nakken 1983; Knudsen 1989) the operator was required to identify the schools by eye, using either the paper read-out or stored data from the sounder. He then had to quantify the echo energy associated with each school. In our system, this has already been determined by the computer. This accurate matching of the echo to the school avoids a number of problems associated with previous methods. As only the echo from the actual school is used, there is less need to compensate for the presence of plankton in any particular integration layer. Additionally, a large number of small schools with relatively weak echoes may be difficult to quantify manually from the integrator plots whereas the system described here should be able to accurately quantify a large number of very small schools.

As well as recording the echo energy of each specific school, the system can also record a wide variety of physical parameters related to that school. This information can be utilised both to improve the efficiency of the stock estimate and to study the pattern of fish distribution. One of the major problems with echo-integration is the assignment of a particular school type to a particular species or species combination. This must necessarily be a subjective interpretation based on the operator's experience and the evidence from adjacent trawls. The collection of physical parameters associated with every school, within any one survey, allows the operator to determine whether these interpretations have remained consistent throughout the survey. Additionally, the assembly of a database of school structure (shape, position, etc.) in relation to species composition (based on trawl data) may, in the future, allow the development of algorithms to advise the operator on the most likely species composition of a particular school based on the earlier identifications in that survey, and possibly previous surveys.

The collection of this type of data on each school also allows the fisheries scientist to study the problem of fish distribution in much more detail. For the first time, it will be possible to study the statistics of fish distribution at the scale of normal fish aggregations, and not at an artificial scale, imposed by the echo-integrator. This information will be of use in studying the statistics of survey design and stock assessment. The analysis may need to be different for fish concentrated in a few large schools rather than many small ones. Alterations of schooling pattern on a daily, seasonal, or geographic basis may also affect the way in which surveys are designed and analysed. Nonlinear effects in dense schools (Foote 1990; Toresen 1991) may also be studied more easily and systematically after the precise delineation of school structure and density.

A similar method for extracting morphological and positional parameters of fish schools for acoustic data has been described by Souid (1987) and Masse and Rouxel (1991). Although the technique for locating the schools is not described, the methods for acquiring the school parameters are very detailed and the differences between the two approaches need to be considered. Prior to analysis, Souid (1987) applied a correction to the data to produce pixels that are approximately square, i.e., the vertical and horizontal dimensions are the same. While this has the advantage of making calculations on the geometry of the image easier, it does require the averaging of adjacent samples, with concomitant loss of resolution. It is possible to determine all the parameters that we have chosen to study without this correction. We have not, therefore, made this correction in our data. After applying this correction, Souid made no attempt to correct the school dimensions for either pulse length or beam angle. After correction to produce square pixels, Souid analysed the data using a method based on the invariant moments of the image (Hu 1962; Duda and Hart 1973). This technique has the apparent advantage of allowing the extraction of school morphology functions, which are unaffected by scale or orientation. We believe that the removal of orientational information is probably undesirable. In illustration, an elliptical school, with its longest axis vertical, is clearly different from one with an identical shape, but with the longest axis horizontal. The different orientations may reflect any number of biological differences including the behaviour, species, or age of fish. The use of invariant moments may be useful, and we are currently examining this approach, but it is also very important to retain information about the orientation of the schools.

In a more general application, it should be possible using this method to study the schooling behaviour of fish in the wild in more detail than has hitherto been possible. We may be able to relate different school structures with different times of day (Azzali et al. 1985), different types of bottom features (Yamaguchi et al., 1984), or different age–length groups. Migratory fish, such as herring or mackerel, may adopt different schooling patterns depending on whether they are feeding or actively migrating.

The image analysis and object labelling system described here can handle objects of any size or shape within the image. It would be straightforward to adapt the design to different types of aggregations, e.g., fish or plankton layers.

In conclusion, this paper outlines a new, very rapid, and efficient technique for the detailed analysis of acoustic survey data. It can produce data on a number of physical and positional parameters of fish schools and should be able to substantially improve the accuracy of some aspects of acoustic stock estimates. Such analysis to date has been either impossible or very tedious.

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Environmental Control of Green Sea Urchin, *Strongylocentrotus droebachiensis*, Spawning in the St. Lawrence Estuary[1]

Michel Starr[2] and John H. Himmelman

Département de biologie and GIROQ, Université Laval, Québec (Québec), Canada G1K 7P4

and Jean-Claude Therriault

Institut Maurice-Lamontagne, Ministère des Pêches et des Océans, C.P. 1000, Mont-Joli (Québec), Canada G5H 3Z4

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Environmental factors and spawning of the green sea urchin, *Strongylocentrotus droebachiensis*, were examined during 1983 and 1984 in the St. Lawrence Estuary. In both years, spawning occurred in June, which contrasts sharply with the February to early May spawnings reported for other locations. This difference does not appear to be related to temperature, but to the much delayed spring increase of phytoplankton in the Estuary. In both 1983 and 1984, sea urchin spawning coincided with the first marked and sustained increase in phytoplankton abundance (chlorophyll *a* levels of 1–2 $mg \cdot m^{-3}$ for >3 d) which took place about 2 wk before the main spring bloom; this first increase coincided with a sharp increase in salinity resulting from a decrease in freshwater runoff. Sea urchin spawned later in 1983 than in 1984. This difference did not appear to be related to either a critical temperature level or short-term temperature fluctuations; however, it did coincide with a greater freshwater runoff and a consequently delayed seasonal increase in salinity and phytoplankton. We hypothesize that onset of the phytoplankton bloom initiates spawning; freshwater runoff may indirectly determine the time of spawning by controlling when the bloom will occur.

Nous avons examiné en 1983 et 1984, dans l'estuaire du Saint-Laurent, les facteurs environnementaux et la ponte de l'oursin vert, *Strongylocentrotus droebachiensis*. Les deux années, la ponte a eu lieu en juin, alors qu'on l'a observée beaucoup plus tôt ailleurs, de février au début de mai. Cette différence ne semble pas liée à la température, mais plutôt au fait que la prolifération du phytoplancton dans l'estuaire est très retardée. En 1983 comme en 1984, la ponte de l'oursin vert a coïncidé avec la première augmentation marquée et durable de l'abondance du phytoplancton (concentration de chlorophylle *a* de 1 à 2 $mg \cdot m^{-3}$ pendant plus de trois jours) qui a été observée environ 2 sem avant la principale efflorescence printanière; cette première poussée coïncidait avec une forte augmentation de la salinité causée par une diminution de l'apport d'eau douce. Les oursins ont frayé en 1983 plus tard qu'en 1984. Cette différence ne semblait liée ni à un niveau critique de température ni à des fluctuations thermiques à court terme; elle coïncidait toutefois avec un volume plus important d'eau douce, ce qui a retardé l'augmentation saisonnière de la salinité et la poussée phytoplanctonique. Nous posons l'hypothèse que le début de la poussée phytoplanctonique fait démarrer la ponte; l'apport d'eau douce peut indirectement fixer le moment de la ponte en déclenchant l'efflorescence.

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Spawning in marine invertebrates is widely thought to be controlled by environmental factors (Giese and Kanatani 1987). This should ensure that the early developmental stages encounter favorable conditions and that gamete release is synchronized within populations, thereby enhancing fertilization success. In examining mechanisms controlling spawning, it is useful to distinguish proximate spawning factors, the environmental changes that trigger gamete release, from ultimate factors that exert a selective pressure on the timing of spawning (Baker 1938). Many environmental factors have been suggested as proximate spawning signals. These include temperature (Loosanoff and Davis 1963; Galtsoff 1964; De Wilde and Berghuis 1978), phases of the moon (Korringa 1947; Mileikovsky 1958; Hauenschild 1960; Iliffe and Pearse 1982), light (Lambert and Brandt 1967; Georges 1968; Woollacott 1979), salinity (Panikkar and Aiyar 1939; Wilson 1969; Stephens and Shetty 1981), phytoplankton abundance (Himmelman 1981; Smith and Strehlow 1983), food abundance (Barnes 1957; Booloottian 1966; Hines 1978), waves (Young 1945), desiccation (Calisle 1962), and chemical substances (Morse et al. 1977; Fitt and Trench 1981). However, in spite of the vast literature on reproduction in marine invertebrates, most considerations about proximate spawning factors remain highly speculative (Himmelman 1981; Giese and Kanatani 1987).

The poor understanding of the control of spawning is due to a lack of detailed records of environmental conditions during

[1]Contribution to the programs of GIROQ (Groupe Interuniversitaire de recherches océanographiques du Québec) and of the Maurice Lamontagne Institute (Department of Fisheries and Oceans, Biological Oceanography Division).

[2]Present address: Institut Maurice-Lamontagne, Ministère des Pêches et des Océans, C.P. 1000, Mont-Joli (Québec), Canada G5H 3Z4.

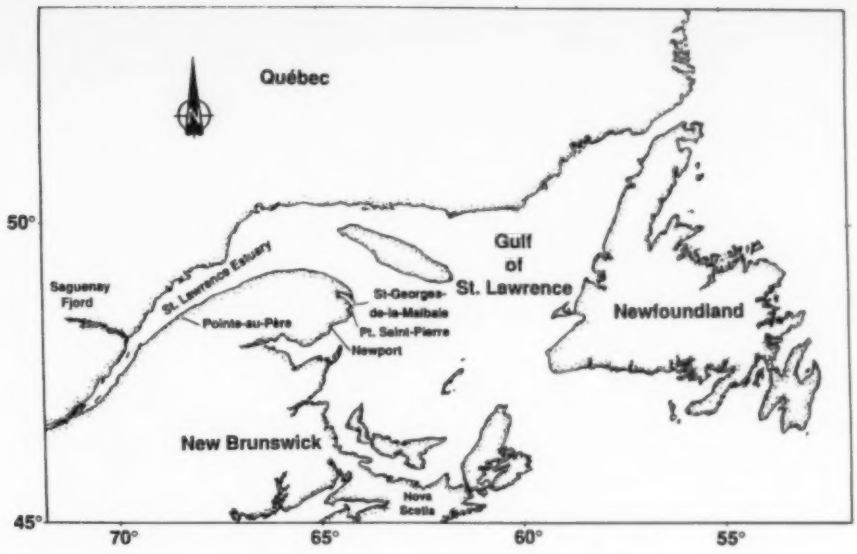

FIG. 1. Map of eastern Canada showing collection sites.

spawning and a lack of experimental studies. Correlative analyses are usually limited to the most readily measurable variables, and the frequent implication of temperature as a proximate spawning signal may reflect the absence of data on other potential factors.

In recent laboratory studies, we demonstrated that heat-stable metabolities released by various species of phytoplankton induce spawning in the green sea urchin, *Strongylocentrotus droebachiensis* O.F. Müller, and the blue mussel, *Mytilus edulis* L. (Starr et al. 1990). Since phytoplankton concentrations sufficient to induce spawning are usually attained during spring blooms, we hypothesize that phytoplankton triggers spawning, and thus that spawning is directly coupled with the food source of the larvae.

In the present study, we examine the relationship between spawning in the sea urchin *S. droebachiensis* and environmental conditions in the St. Lawrence Estuary, eastern Canada. Conditions in the St. Lawrence Estuary contrast sharply with those in locations where previous spawning observations have been made for this species. The St. Lawrence is a large and partially mixed estuary, showing distinct seasonal as well as short-term variability in oceanographic characteristics associated with semidiurnal and fortnightly tidal cycles (Demers et al. 1986). Moreover, the spring phytoplankton bloom is markedly delayed compared with other regions at the same latitude (Sinclair 1978; Sinclair et al. 1981; Levasseur et al. 1984; Therriault and Levasseur 1985, 1986). Thus, the study of the relationship between spawning of sea urchins and environmental factors in the St. Lawrence Estuary may improve our understanding of mechanisms controlling invertebrate spawning.

Materials and Methods

We examined spawning in sea urchins near the INRS-Océanologie laboratory wharf at Pointe-au-Père, near Rimouski (Fig. 1). Samples for gonadal index determination were collected frequently by SCUBA divers during the spring and summer of 1983 and 1984 at 2–5 m depth. To avoid variations in the gonadal index due to animal size (Gonor 1972), only sea urchins 4–6 cm in diameter were used. The percentage of total weight contributed by gonads was used as the gonadal index for both male and female sea urchins. All wet weight determinations were recorded to the nearest 0.01 g after removal of surface moisture. For each female, a rapid smear was made of a fresh ovary to determine the percentage of mature oocytes. Under the microscope, 100 oocytes were examined from each sea urchin. Oocytes were considered mature if they had a dense yolk-filled cytoplasm, a chorion, a regular rounded shape, and a diameter >80 μm (Stephens 1972).

Phytoplankton biomass was quantified using chlorophyll *a* determinations carried out in duplicate on water samples taken with a Niskin bottle from 3 m depth at a fixed station, located ~50 m offshore. Samples were always collected at high tide to reduce variability due to tidal effects. For each sample, 100 mL of water was filtered onto a Whatmann GF/C glass fiber filter which was stored frozen with silica gel for later fluorometric analysis. The 90% acetone extraction method of Yentsch and Menzel (1963), as modified by Holm-Hansen et al. (1965), was used.

Temperature and salinity were recorded daily from seawater pumped into the INRS-Océanologie laboratory. The intake was located ~5 m below the low water level near the collection site. Daily means were calculated from four to nine measurements.

The relationships among daily variations of temperature, salinity, tidal amplitude, and chlorophyll *a* were examined from 5 May to 24 July during both years using a Kendall rank correlation analysis (Sokal and Rohlf 1981). To remove the effect of seasonal trends in the physical variables, correlation coefficients were computed from the residuals between daily observed values and the seasonal trend as calculated by the least squares method (Legendre and Legendre 1984). A linear relationship was used for temperature data whereas a third-order relationship was used for the salinity series. Chlorophyll *a* data for May and June, when chlorophyll *a* levels were low, were examined separately from those for July, when concentrations were high. Temporal variations of gonadal and maturity indices

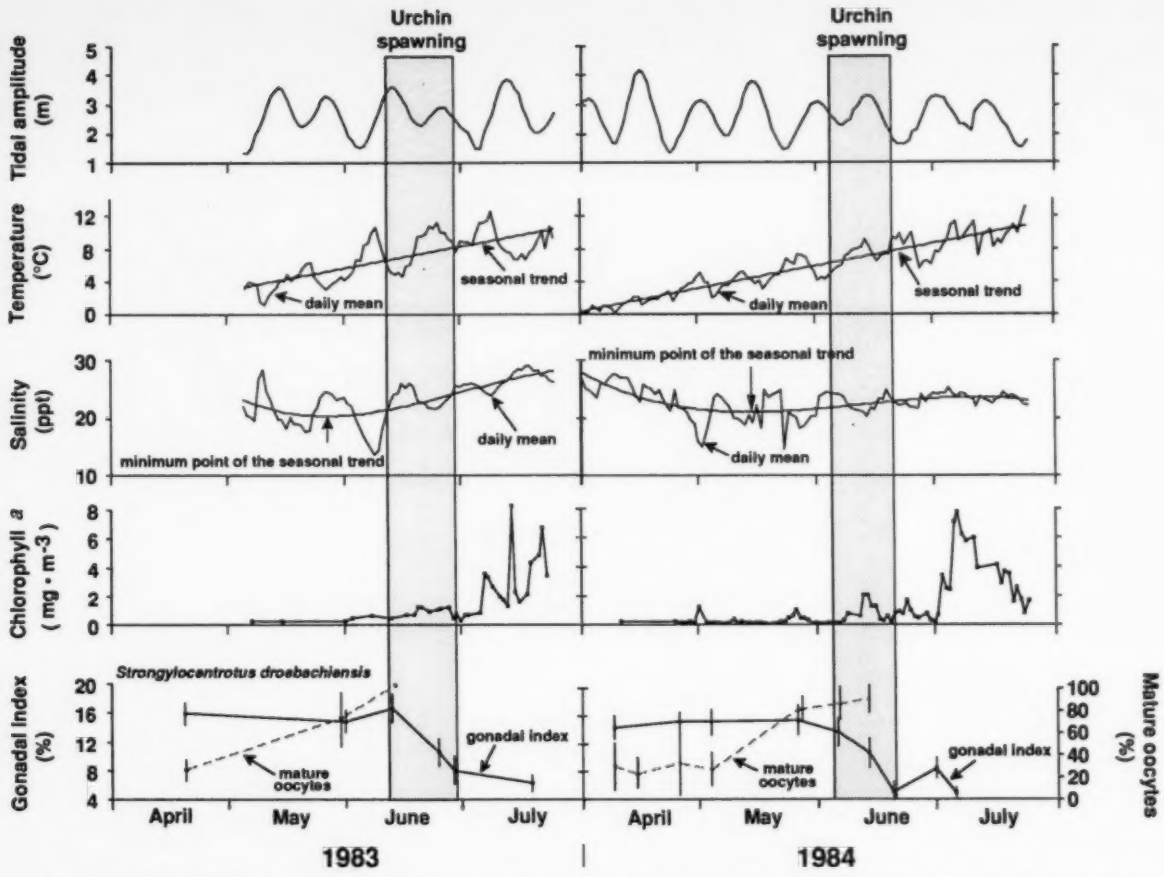

FIG. 2. Temporal variations in the mean gonadal index and the percentage of mature oocytes for the green sea urchin *S. droebachiensis* (n = 30) in relation to tidal amplitude, daily mean temperature and salinity, and chlorophyll *a* concentration at Pointe-au-Père, St. Lawrence Estuary, during the spring and summer of 1983 and 1984. Vertical lines indicate the 95% confidence intervals of gonadal index values.

were analyzed using ANOVA and Scheffe tests, and the data were normalized using arc sine transformations (Sokal and Rohlf 1981) prior to these tests.

Data on freshwater runoff during the study period were provided by Environment Canada (Climatologic Services). We used the combined discharges from the upper Estuary, the Saguenay Fjord, and from the rivers of the lower Estuary upstream of Pointe-au-Père. Tidal amplitude data were obtained from the published tables of the Canadian Hydrographic Service (Department of Fisheries and Oceans, Ottawa, Canada).

Results

Temperature and Salinity

Winter conditions, with temperatures <0.5°C and salinites ≈27‰, were present when sampling began in early April 1984 (Fig. 2) During subsequent sampling, the general temperature pattern, excluding short-term tidal variations, showed an almost linear increase to the annual maximum of ~10°C in late July. In contrast, surface salinity showed a general decrease during May, coinciding with peak freshwater runoff (Fig. 3), followed by an increase during June to the summer maximum. In addition to the general seasonal patterns, temperature and salinity showed distinct short-term cycles associated with the fortnightly tidal cycle (Fig. 2). Throughout the sampling period, there was a strong negative correlation between temperature and salinity (Kendall $t = -0.436$, $P < 0.01$). In April, high temperatures and low salinities coincided with spring tides (Fig. 2). Subsequently, there was a progressive shift in this relationship until July, when periods of high temperatures and low salinities

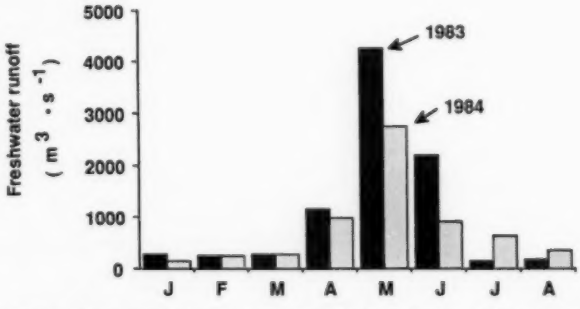

FIG. 3. Mean monthly freshwater runoff from April to July in 1983 and 1984.

approximately coincided with neap tides (Fig. 2). As a result of this shift, temperature cycles were not significantly correlated with tidal amplitude for the entire sampling period (Kendall $t = 0.088$, $P > 0.05$), although these factors were significantly correlated for the period from 5 May to 24 July (Kendall $t = -0.196$, $P < 0.05$). The changes in relationships between the temperature and salinity cycles and the tidal cycle were probably related to freshwater input. This type of seasonal transition seems typical of the St. Lawrence Estuary (Sinclair 1978; De Lafontaine et al. 1984). Between 5 May and 24 July, the tidal cycle and the cycles in physical variables were more or less in phase, probably reflecting interactions between the freshwater outflow and tidal forces.

In 1983, the general warming pattern was nearly identical to that in 1984 (Fig. 2; regression curves for the two years were not significantly different; *t*-test, $P > 0.05$). In contrast, surface salinity showed different patterns in the two years. Following the decrease during April–May, the seasonal increase occurred ~2 wk later in 1983 than in 1984 (Fig. 2; in 1984, the minimum point of seasonal trend occurred on 14 May whereas in 1983, this was observed on 27 May). This delay was probably due to the greater freshwater outflow in 1983 (Fig. 3). Finally, the variance around seasonal temperature trend showed that the short-term temperature variations were greater in 1983 than in 1984 (Fig. 2).

Chlorophyll *a*

In 1984, low phytoplankton biomass, characteristic of the winter, persisted until late May, except for two short pulses (<3 d) in which chlorophyll *a* concentrations reached ~1 $mg \cdot m^{-3}$ (Fig. 2). Chlorophyll *a* concentrations were higher

TABLE 1. Relationship between spawning time of the green sea urchin *S. droebachiensis* and (1) the temperature attained during spawning and (2) the timing of the spring phytoplankton bloom in different geographical regions.

Locality	Spawning time	Temperature	Spring phytoplankton bloom
St. Lawrence Estuary			
Pointe-au-Père	June (present study)	4–10°C (present study)	Starts in June, maximum in July (present study)
Gulf of St. Lawrence			
Pt. Saint-Pierre and Newport	Finished in May (present study)	<4°C (at Grande Rivière) (Bugden et al. 1982)	Late April to early May (Steven 1974)
St-Georges-de-la-Malbaie	Late April to early May (present study)	<4°C (at Grande Rivière) (Bugden et al. 1982)	
Newfoundland			
Portugal Cove	March to April (Himmelman 1978)	<3°C (Himmelman 1969)	April (Himmelman 1978)
Nova Scotia			
St. Margaret's Bay	April (Miller and Mann 1973)	2–4°C (Miller and Mann 1973)	April (Platt and Irwin 1970; Platt 1971)
Northeastern United States			
Lamoine, Maine	April (Cocanour and Allen 1967)		Starts in April, sometimes in May (Bigelow et al. 1940)
Salisbury Cove, ME	April to mid-May (Harvey 1956)		
Boothbay Harbor, ME	Early April (Stephens 1972)	8°C (Taylor et al. 1957)	Starts in mid- to late March (Bigelow et al. 1940)
Cape Cod, MA	Late April (Stephens 1972)		Starts in late March to third week of April (Bigelow et al. 1940)
Woods Hole, MA	March (Boolootian 1966)	1–2°C (Taylor et al. 1957)	Midwinter bloom (Fish 1925)
British Columbia			
First Narrows	April (Himmelman 1976)	6–8°C (Himmelman 1976)	April (Himmelman 1976)
Botanical Beach	April (Himmelman 1976)	8–9°C (Himmelman 1976)	Mid-April (Himmelman 1976)
Norway			
Bergen	Late March (Runnström 1927a, 1927b)	4–5°C (Brown 1984)	Starts in mid-March, maximum between March and April (Gran 1928; Gaarder 1938; Sakshaug and Myklestad 1973)
Trömso	February to March (Vasseur 1952)	2°C (Brown 1984)	
Tromsösundet	March (Falk-Petersen and Lonning 1983)		
White Sea	Mid-June to mid-July (Kaufmann 1974)	3–5°C (Kaufmann 1974)	

between June 7 and 30, occasionally up to 2 $mg \cdot m^{-3}$, indicating a distinct increase in phytoplankton biomass. An intense bloom occurred in early July. Each phytoplankton pulse prior to the major bloom coincided with a period of increased temperature and decreased salinity (Fig. 2; Kendall t between chlorophyll and temperature and between chlorophyll and salinity equal to 0.469 and -0.363 respectively, $P < 0.01$). In 1983, chlorophyll *a* concentration increased from winter values of ~0.1 $mg \cdot m^{-3}$ in May to ~0.5 $mg \cdot m^{-3}$ in early June to ~1 $mg \cdot m^{-3}$ in late June and finally to much higher levels in July (Fig. 2).

Reproductive Cycle of *S. droebachiensis*

Significant decreases in the mean gonadal index in the population of sea urchins at Pointe-au-Père indicated that spawning occurred in June in both 1983 and 1984 (Fig. 2). However, spawning appeared to be later in 1983 than in 1984. In 1984, the first significant decrease in the gonadal index occurred between 7 and 13 June (an ANOVA shows heterogeneity among the samples, $P < 0.001$, df = 9, and a Scheffe F-test identifies the 13 June sample as being different from indices in April and May) whereas in 1983, the first significant drop occurred between 12 and 29 June (Scheffe F-test, $P < 0.05$). The later spawning in 1983 was confirmed by visual examination of the gonads; 80% of the sea urchins were completely spent on 19 June 1984 (the gonads were dark in colour, had indices of <6.0, and gametes did not leak from the tissues when the gonad was cut) compared with 13% on 24 June 1983. By 18 July 1983 the proportion of spent urchins (63%) approached that observed on 19 June 1984.

Although in both years, gonadal indices were near maximum when sampling began in the spring (Fig. 2), an active period of vitellogenesis occurred in May as evidenced by an increase in the percentage of mature oocytes from <30 to 70–80% (ANOVA followed by Scheffe F-test, $P < 0.05$). Since in both years, the percentage of mature oocytes was high in late May, the later spawning period in 1983 could not be attributed to differences in the timing of gonadal maturation. Furthermore, spawning did not appear to occur spontaneously after gonadal maturation, since concurrent laboratory studies showed that no spawning occurred when mature sea urchins were maintained for more than 1 mo under simulated natural conditions (temperature of 7°C, 21–27‰ salinity, 16 h light : 8 h dark photoperiod; Starr 1990).

Discussion

Environmental Control of Sea Urchin Spawning

Temperature

The emphasis on temperature as a proximate spawning cue became prominent following Orton's (1920) postulate that a critical temperature level stimulated breeding activity. A corollary of this hypothesis, that spawning should occur earlier at low latitudes and progressively later at higher latitudes, appears to be supported by data for a number of invertebrates in the North Atlantic (Runnström 1927a; Fenaux 1968; 1970; Mileikovsky 1970a, 1970b; Sastry 1970; Seed 1976). However, the results of our study do not indicate that spawning of the green sea urchin is triggered by a critical temperature level. The June spawning in the St. Lawrence Estuary is much later than in other areas of the North Atlantic and North Pacific, where spawning occurs between February to early May

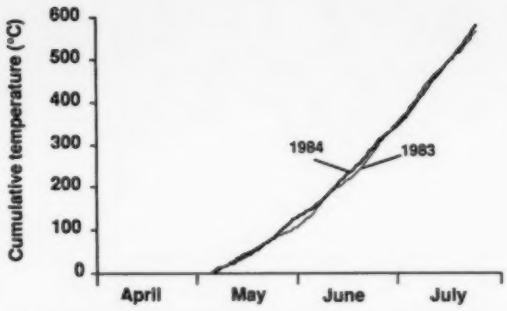

FIG. 4. Cumulative temperature (degree-days calculated from 5 May to 23 July) at Pointe-au-Père, St. Lawrence Estuary, during 1983 and 1984.

(Table 1), at the beginning of the vernal warming period. Temperatures at the time of spawning in the St. Lawrence Estuary vary in the range of 4–10°C in contrast with temperatures <4°C at the other locations in the North Atlantic. There is also no clear trend between sea urchin spawning and temperature for the previously reported spawning data (Table 1). Spawning temperatures vary markedly over short as well as long geographical distances. The only available data that might indicate a delay with increasing latitude are those for the White Sea in the Arctic Ocean, where spawning occurs in July (Kaufmann 1974). Furthermore, the unusual spawning time in the St. Lawrence Estuary, as compared with other populations for the North Atlantic and North Pacific does not appear to be related to the annual temperature regime. For example, the annual cumulative temperature in the St. Lawrence Estuary (estimated from data of Therriault and Levasseur 1985) is ~1200°C-days, only slightly less than that at Tromsö, Norway (~1400°C-days; Brown 1984), where spawning occurs in February–March (Vasseur 1952).

An alternative hypothesis is that the varying spawning response to temperature in different regions is due to distinct physiological races (such as those described for *Crassostrea virginica* and *Aequipecten irradians*; Loosanoff and Nomejko 1951; Sastry 1970). This implies genetic differences among populations. However, our data for the Estuary suggest that this hypothesis does not hold for *S. droebachiensis*. Although the general warming and cumulative temperature patterns at Pointe-au-Père were virtually identical in 1983 and 1984 (Fig. 2 and 4), spawning was later in 1983. The temperature had reached 10°C before spawning began in 1983 and was 7–8°C during the early June spawning in 1984 (Fig. 2).

Many studies suggest that temperature fluctuations stimulate spawning (e.g., Young 1945, De Wilde and Berghuis 1978). Our data, however, do not indicate a consistent relation between sea urchin spawning and the short-term temperature fluctuations caused by the tidal spring-neap tide cycle. Spawning occurred later in 1983 even if greater short-term temperature variations occurred earlier than in 1984 (Fig. 2). Neither can the difference in spawning time be attributed to a delay in gonadal maturity, since in both years, the urchins were ready to spawn (>70% of the oocytes mature) by late May (Fig. 2).

Salinity

Spawning coincided with the seasonal increase in salinity during both years of our study. Thus, the late spawning period in 1983 compared with 1984 coincided with the later salinity increase caused by greater freshwater runoff (Fig. 3). Wilson

(1969) described a similar relationship between spawning and increasing salinity for an estuarine population of the mussel *Xenostrobus securis* in Australia and suggested that a wave of spawning moves up the estuary as salinities reach a level suitable for larval development. In the Gulf of St. Lawrence, sea urchin spawning occurs much earlier than in the Estuary (Fig. 1; Table 1). We documented a significant drop in the gonadal index due to spawning between late April and early May 1985 at St-Georges-de-la-Malbaie (an ANOVA showed a heterogeneity among the samples of 20–50 sea urchins taken on 10 April, 26 April, and 6 May and a Scheffe F-test showed that the 6 May indices were less than those in April; $P < 0.05$). We also found that sea urchins collected in early May 1978 at Pointe Saint-Pierre and Newport ($n > 30$) had depleted gonads (unpublished data). Thus, the delay in sea urchin spawning in the St. Lawrence Estuary, compared with the Gulf and other more oceanic areas, appears to be related to the low salinity conditions during the spring.

Nevertheless, we consider it unlikely that salinity is the proximate spawning factor because, with the strong fortnightly salinity cycle, sea urchins at Pointe-au-Père could not have detected the seasonal increase in salinity until at least one full tidal cycel was completed. Also, our data do not indicate a consistent relationship between short-term salinity variations and spawning (Fig. 2).

Phytoplankton

It is probable that freshwater runoff affects spawning indirectly through its influence on phytoplankton growth. The bloom in the Estuary is about 2 mo later than in adjacent regions. We attribute this to freshwater runoff which may prevent an accumulation of phytoplankton by reducing its residence time in the Estuary (Malone 1977; Côté and Lacroix 1979; Sinclair et al. 1981) or by decreasing eddy exchange between surface and deeper waters (due to strong stratification), thus preventing the seeding of the surface water with marine phytoplankton cells (Levasseur et al. 1984; Therriault and Levasseur 1985, 1986). Consequently, the phytoplankton bloom is restricted to the Gulf in early spring during high freshwater discharge (Steven 1974) and gradually progresses up the Estuary as freshwater runoff decreases (Therriault and Levasseur 1985, 1986). In the Gulf and other more oceanic areas, sea urchin spawning has been observed in the early spring coinciding with the usual period of the phytoplankton spring bloom (Table 1). In the Estuary, the phytoplankton bloom is markedly delayed and spawning occurs as soon as phytoplankton growth begins (Fig. 2). Thus, spawning occurred between 12 and 29 June in 1983 and between 7 and 13 June 1984 when chlorophyll *a* concentrations first reached 1.3–2 $mg \cdot m^{-3}$ for a period >3 d. Chlorophyll *a* levels prior to these periods were very low.

In this situation, if phytoplankton was the spawning cue, the threshold was a chlorophyll *a* concentration of 1–2 $mg \cdot m^{-3}$ sustained for> 3 d. Our previous laboratory study demonstrated that the spawning response of the sea urchin is dependent upon the concentration of chlorophyll *a*, and a ~20% spawning response is obtained when the urchins are exposed to a concentration of 2 $mg \cdot m^{-3}$ for a period of 7 d (Starr et al. 1990). Since sperm suspension strongly stimulates spawning (Starr et al. 1990), increased spawning would be expected once spawning was initiated. It should be noted that, although offshore and inshore phytoplankton abundances vary in parallel in the Estuary, inshore values are much lower, by as much as 10-fold during June (De Lafontaine et al. 1984). Grazing by benthic filter feeders may contribute to this reduction (Demers et al. 1989). By diffusion, extracellular substances (Starr et al. 1990) from offshore phytoplankton should further intensify spawning.

To summarize, sea urchin spawning in the St. Lawrence Estuary does not appear to be related to temperature, but coincides with the salinity increase that follows the spring runoff. It seems unlikely that spawning is controlled by salinity changes, but it may be triggered by the phytoplankton increase that follows the decrease in freshwater outflow. Thus, we hypothesize that onset of the bloom initiates spawning and that this and the additive stimulus of sperm (and possibly other secondary stimuli such as turbulence and short-term variations of temperature) lead to epidemic spawning. Further work with a long time-series of sea urchin spawning and environmental factors data is required to confirm this hypothesis.

Strongylocentrotus droebachiensis larvae begin feeding 1–2 wk after fertilization (Stephens 1972) and the pelagic phase lasts >50 d (Strathmann 1978). Thus, spawning at the onset of the bloom should permit larvae to attain the planktotrophic stage near the peak of the bloom, and the larvae should benefit maximally from the bloom. Since freshwater runoff in the St. Lawrence Estuary decreases preceding the spring phytoplankton bloom, the coupling of spawning with the bloom should result in favorable salinities for the larvae as well as increased larval retention.

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In situ Bioassays of Brook Trout (*Salvelinus fontinalis*) and Blacknose Dace (*Rhinichthys atratulus*) in Adirondack Streams Affected by Episodic Acidification

Howard A. Simonin

New York Department of Environmental Conservation, 8314 Fish Hatchery Road, Rome, NY 13440, USA

Walter A. Kretser

New York Department of Environmental Conservation, P.O. Box 296, Ray Brook, NY 12977, USA

and Dale W. Bath, Mark Olson, and James Gallagher

Adirondack Lakes Survey Corporation, P.O. Box 296, Ray Brook, NY 12977, USA

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In situ bioassays were conducted using native Adirondack brook trout (*Salvelinus fontinalis*) and blacknose dace (*Rhinichthys atratulus*) in four headwater streams. Conductivity, pH, temperature, and stage height were monitored continuously, and water samples for laboratory analysis were collected during hydrologic episodes. Fish survived well during baseflow conditions, but during periods of spring snowmelt or large precipitation events, survival was poor. Blacknose dace were more sensitive than brook trout, and mortality was best correlated with the log of median inorganic monomeric aluminum (Al_{im}) concentration. Brook trout mortality was best correlated with a two-variable model that included dissolved organic carbon (DOC) and a concentration–duration variable (median Al_{im} during the episode times the duration of the episode). Brook trout mortality was inversely correlated with DOC. Bioassay fish that had been in the streams 15–24 d survived episodes better than fish that had either not become acclimatized or recovered from handling. Duration of exposure to acidic episodes was critical. Extended periods of poor water quality resulted in fish mortality and may be more important to native populations than short acidic episodes.

Des dosages biologiques in situ ont été réalisés avec des ombles de fontaine (*Salvelinus fontinalis*) et des naseux noirs (*Rhinichthys atratulus*) indigènes d'Adirondack dans quatre ruisseaux d'amont. La conductivité, le pH, la température et le niveau de l'eau ont fait l'objet d'une surveillance continue et des échantillons d'eau ont été prélevés pour analyse de laboratoire au cours des épisodes hydrologiques. Les poissons ont eu une bonne survie durant les conditions de débit de base, mais durant la fonte des neiges printanière et les grandes précipitations, la survie a été médiocre. Le naseux noir s'est révélé plus sensible que l'omble de fontaine et la mortalité présentait la meilleur corrélation avec le log de la concentration médiane d'aluminium monomère inorganique (Al_{im}). Chez l'omble de fontaine, la mortalité présentait la meilleure corrélation avec un modèle à deux variables, à savoir la concentration de carbone organique dissous (COD) et une variable concentration–durée (Al_{im}) médiane au cours de l'épisode multiplié par la durée de l'épisode). La mortalité de l'omble de fontaine présentait une corrélation inverse avec la COD. Les poissons ayant séjourné dans les ruisseaux pendant 15–24 jours ont mieux survécu aux épisodes que les poissons qui n'avaient pas eu le temps de s'acclimater ou qui ne s'étaient pas rétablis de la manipulation. La durée de l'exposition à des épisodes où l'eau est plus acide était critique. Des périodes prolongées où la qualité de l'eau était médiocre ont entraîné de la mortalité et pourraient avoir plus d'importance pour les populations indigènes que les courtes périodes où l'acidité est élevée.

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Both laboratory and field bioassays have been used to demonstrate that fish are sensitive to acid and high aluminum concentrations (Baker and Schofield 1982; Sharpe et al. 1983; Johnson et al. 1987; Parkhurst 1987; Ingersoll et al. 1990). Relatively few studies have been conducted to evaluate the effects of episodic acidification that occurs during periods of snowmelt or heavy precipitation. Such episodes occur commonly in streams and rivers receiving acidic deposition and may vary in both intensity and duration. Following these episodes, water quality typically returns to conditions acceptable to most fish life in all but chronically acidified streams. Detailed reviews of the effects of acidic episodes have been prepared by Marmorek et al. (1986) and Wigington et al. (1990).

Episodic acidification in the Adirondack region of New York State has been documented in lake outlets (Driscoll and Newton 1985; Galloway et al. 1987), large rivers (Colquhoun et al. 1981, 1984), and headwater streams (Colquhoun et al. 1982, 1984; Colquhoun and Aylesworth 1983). The most acidic epi-

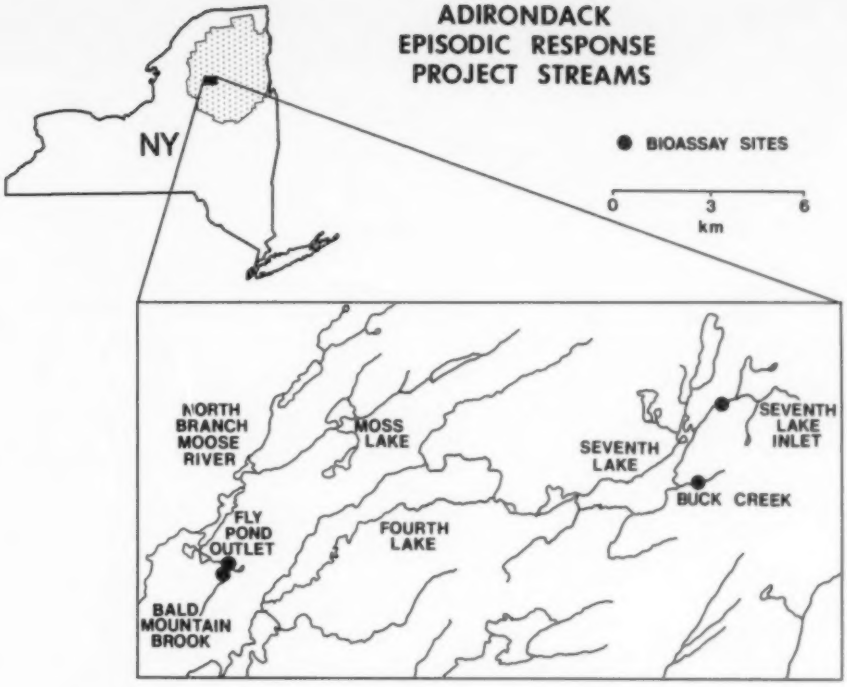

FIG. 1. Location of Adirondack stream bioassay sites.

sodes of longest duration generally occur during spring snowmelt, although episodes equal in acidity and duration may also occur at other times of the year, depending on the amount of precipitation.

Fisheries survey data have shown that fish are absent from many Adirondack streams that become acidic during the spring snowmelt period (Colquhoun et al. 1983). Schofield and Driscoll (1987) found that in the North Branch of the Moose River, acidity gradients in the watershed and the acid tolerance of the fish species were the key determinants in the species distributions they observed. Johnson et al. (1987) further suggested that the extent of the watershed that is acidic, and therefore toxic to fish, increases during snowmelt and major runoff events.

Studies in Pennsylvania (Gagen and Sharpe 1987; Sharpe et al. 1987) and in Norway (Hesthagen 1989) demonstrated fish mortalities in response to acidic episodes. During baseflow conditions, streams offered suitable fish habitat and water quality, but during spring snowmelt, poor water quality resulted in fish mortalities. Hesthagen (1989) counted dead fish daily in a Norwegian river and associated the cause of death with environmental acidification during spring snowmelt. He also observed that fish mortality was usually delayed relative to the onset of an acidic episode. Gunn (1986) concluded that acidic episodes may impact several species of salmonids at different periods in their life history. He also noted that few field studies had been conducted on the impacts of acidification on brook trout (*Salvelinus fontinalis*) populations.

The Adirondack Episodic Response Project was designed to address fish response during episodic acidification. This project was part of a larger effort coordinated by the U.S. Environmental Protection Agency with similar research conducted in Pennsylvania and in the Catskill Mountains of New York State. Several objectives of the Adirondack project were to (1) continuously monitor the water chemistry of several streams that experience episodic acidification, (2) determine the effects of acidic episodes on the survival of brook trout and blacknose dace (*Rhinichthys atraulus*) by conducting in situ bioassays, and (3) relate the bioassay results to fish population responses observed in the study streams. Additional components of the Adirondack Episodic Response Project are discussed in Kretser et al. (1991).

Methods

Four headwater streams were selected as study sites for the Adirondack Episodic Response Project (Fig. 1). The streams are located in the southwestern Adirondack region which receives over 120 cm of precipitation per year. Fly Pond Outlet and Bald Mountain Brook are in the North Branch of the Moose River drainage. Seventh Lake Inlet and Buck Creek are tributaries of Seventh Lake and are in the Middle Branch of the Moose River drainage. The watersheds are covered by state forest consisting of a northern hardwood/conifer mixture.

Conductivity, pH, temperature, and stage height of the streams were measured continuously. An instrument shelter was constructed at each stream to house a U.S. Geological Survey minimonitor, a Campbell (model CR10/WP) data logger/controller, an ISCO (model 2700) automated water sampler, and 12-V batteries. The ISCO sampler was programmed to collect 1-L samples at specified intervals based on changes in stream stage. Weekly grab samples were also collected from the streams near the instrument shelters. Water samples were analyzed for the following parameters: pH, acid-neutralizing capacity (ANC), specific conductance, Cl, NO_3, SO_4, Na, K, Ca, Mg, SiO_2, dissolved organic carbon (DOC), dissolved inorganic carbon (DIC), NH_4, total dissolved aluminum (Al_{td}), total

monomeric aluminum (Al_{tm}), and organic monomeric aluminum (Al_{om}). Inorganic monomeric aluminum (Al_{im}) was calculated by subtracting Al_{om} from Al_{tm}. Kretser et al. (1991) presented a more complete discussion of water chemistry methods and quality control procedures.

Bioassays were conducted four times during the study: fall 1988 (November 2 – November 19), spring 1989 (May 8 – June 15), fall 1989 (September 27 – October 31), and spring 1990 (May 1 – May 29). They were scheduled to coincide with times when acidic episodes generally occur and to coincide with concurrent intensive studies on fish movement in response to episodes (Kretser et al. 1991).

Native brook trout young-of-the-year and native blacknose dace adults were used for the fall bioassay experiments. Native yearling brook trout and blacknose dace adults were used for the spring bioassays. In addition, hatchery-reared (New York Department of Environmental Conservation, Rome Fish Hatchery) brook trout fry were also tested during the spring bioassays. The native brook trout and blacknose dace were collected from over 20 Adirondack streams with water chemistry comparable with that of the study streams. The fish were collected by electrofishing and held for several days in a large cage in Fly Pond Outlet prior to being used for the bioassays. At least 20 fish of each species were tested at each site during each bioassay.

The methods used for fish exposure in the study streams were those used by Johnson et al. (1987). The experimental fish were placed in 3.8-L Nalgene containers with two 7 × 12 cm openings screened with fiberglass (1.4-mm mesh) to ensure adequate water exchange. Three or four containers were used at each site for each species to ensure the fish were not crowded. The containers were then placed inside wood frame cages covered with 6.4-mm mesh plastic screen. The cages served to protect the bioassay containers during periods of high stream flow. The cages were placed in pool areas either adjacent to or near the instrument shelters. The test fish were not fed during the bioassays.

The survival of the bioassay fish was determined by daily checks at each stream. Fish were considered dead if there was no response to prodding and no observed movement. Bioassay experiments were restarted in all streams if greater than 10% mortality occurred in the reference stream (Fly Pond Outlet) or if more than 90% mortality occurred within 1 wk in any stream. As a result of these conditions, three yearling brook trout bioassays were conducted during spring 1989, two blacknose dace bioassays during spring 1989, and two brook trout bioassays during fall 1989.

Analysis of the bioassay and water chemistry data was done primarily with the Statistical Analysis System (SAS). Mortality data were also analyzed as probits (Fisher and Yates 1957) because they often show a sigmoidal relationship to chemical data. The aluminum concentrations were converted to log values prior to regression analysis with mortality in order to improve linearity. Exploratory multiple regression analysis was conducted using the SAS Stepwise Maximum R^2 procedure and the following variables: median field pH, minimum field pH, log(median Al_{td}), log(median Al_{im}), log(peak Al_{im}), median DOC, log(median DOC), median Ca, log(median Ca), and a concentration–duration factor (CONCDUR) defined as the median Al_{im} concentration during an episode (period of daily median $Al_{im} > 100$ μg/L) multiplied by the duration of the episode in days. Daily Al_{im} values were predicted for each stream from the minimonitor pH records and the relationship between minimonitor pH and the measured Al_{im} values. The water chemistry values used were those obtained while each specific bioassay was in progress.

Results

In order to better compare the hydrology of the four study streams, flow data were based on stream watershed areas. Stream baseflow conditions were identified as a daily average flow of 40 $L \cdot s^{-1} \cdot km^{-2}$ or less. Significant hydrologic episodes (over 2.5 times baseflow) occurred during three of the four bioassay periods. The spring 1989 bioassay occurred just following an episode and during a period of gradually decreasing stream flow. During periods of baseflow conditions, water quality in all streams usually allowed for the survival of the bioassay fish. With the exception of Fly Pond Outlet, episodes were generally associated with acidic conditions, higher concentrations of Al_{im}, and higher mortality in the bioassays.

Fly Pond Outlet (the reference stream) exhibited the best water quality of the four study streams. Baseflow pH values averaged 7.1 and during episodes were rarely below 6.0. The hydrologic episodes that occurred in Fly Pond Outlet were not usually associated with water chemistry changes severe enough to cause mortality in the bioassays. Fish mortality and median water chemistry values for selected parameters during the bioassays are presented in Table 1.

Daily Al_{im} concentrations were derived from the continuous record of minimonitor pH using stream-specific predictive equations. This allowed for an evaluation of gradual or small changes in Al_{im} which could be related to the daily observations of fish survival. An average of 80 measured Al_{im} concentrations for each stream were regressed with the corresponding minimonitor pH value for that day to develop the relationship. The following stream specific equations were developed:

Bald Mountain Brook:

$$\log(Al_{im}) = 4.84 - 0.55(pH) \qquad (R^2 = 0.58)$$

Buck Creek:

$$\log(Al_{im}) = 5.27 - 0.65(pH) \qquad (R^2 = 0.66)$$

Seventh Lake Inlet:

$$\log(Al_{im}) = 4.77 - 0.54(pH) \qquad (R^2 = 0.48).$$

Each of these equations was significant at $P < 0.001$. The relationship for Fly Pond Outlet, however, was poor ($R^2 = 0.04$) because pH values were all greater than 5.6 and Al_{im} concentrations were low. The predicted daily Al_{im} concentrations for each stream were then plotted against percent occurrence (Fig. 2). Buck Creek exhibited the highest Al_{im} levels for the greatest percent of the time and Fly Pond Outlet had the lowest Al_{im} levels.

The bioassay that began on May 18, 1989, demonstrated that during periods of low flow, water quality in the study streams is suitable for the survival of blacknose dace. When the 1989 Bald Mountain Brook and Seventh Lake Inlet bioassays are compared with the spring 1990 bioassays for these streams, the effect of the spring 1990 episode is evident (Fig. 3 and 4). Blacknose dace exposed to 50–110 μg Al_{im}/L experienced no mortality, but when an episode began on May 11, 1990, and continued for over 10 d the Al_{im} concentrations increased to over 250 μg/L and resulted in 100% mortality. In Fly Pond Outlet the Al_{im} concentrations remained below 50 μg/L, and no blacknose dace mortality was observed during either 1989 or 1990.

TABLE 1. Selected water chemistry and brook trout and blacknose dace mortality during in situ bioassays.

	Bioassay dates (mo/d/yr)	Median pH	Median Al_{im} (μg/L)[a]	Median DOC (mg/L)	Median Ca (mg/L)	Median Al_{im} during peak (μg/L)[a]	Duration of peak (d)	Mortality (%)
			Brook trout (size range 50–133 mm)					
Bald Mountain Brook	11/2–11/19/88	5.09	111	6.4	2.24	133	12	45
	5/26–6/15/89	5.87	42	3.4	1.96	42	0	50
	9/27–10/9/89	5.87	41	3.5	2.21	100	1	30
	10/6–10/31/89	5.70	51	4.5	2.25	156	5	10
	5/1–5/29/90	4.91	138	4.3	1.83	177	19	65
Buck Creek	11/2–11/19/88	4.67	340	7.5	2.40	340	17	80
	5/26–6/15/89	5.28	70	5.1	2.33	111	3	45
	9/27–10/9/89	4.72	158	5.4	2.40	158	12	50
	10/6–10/31/89	4.89	124	7.4	2.27	199	10	10
	5/1–5/29/90	4.37	283	6.7	2.05	287	25	70
Fly Pond Outlet	11/2–11/19/88	6.70	21	7.8	3.80	21	0	5
	5/26–6/15/89	6.42	24	4.0	4.78	24	0	0
	9/27–10/9/89	6.95	18	2.9	5.75	18	0	15
	10/6–10/31/89	6.65	21	6.4	5.10	21	0	10
	5/1–5/29/90	6.58	22	5.1	4.11	22	0	5
Seventh Lake Inlet	11/2–11/19/88	5.12	210	9.3	3.01	210	17	20
	5/26–6/15/89	5.27	86	6.3	2.51	104	3	0
	9/27–10/9/89	5.47	63	6.7	2.76	108	1	10
	10/6–10/31/89	5.48	62	8.3	2.82	134	5	5
	5/1–5/29/90	4.57	191	7.7	2.32	242	21	25
			Brook trout fry (size range 25–39 mm)					
Bald Mountain Brook	5/9–5/29/89	5.31	85	3.4	1.98	124	8	74
	5/1–5/29/90	4.91	134	4.3	1.83	177	19	27
Buck Creek	5/9–5/17/89	4.79	146	5.4	2.26	146	8	89
	5/1–5/18/90	4.47	233	7.0	2.12	233	17	100
Fly Pond Outlet	5/9–5/29/89	6.38	25	4.1	4.63	25	0	0
	5/1–5/29/90	6.58	22	5.1	4.11	22	0	7
Seventh Lake Inlet	5/9–5/29/89	5.03	111	6.0	2.54	143	15	19
	5/1–5/29/90	4.59	191	7.7	2.32	242	21	0
			Blacknose dace (size range 27–86 mm)					
Bald Mountain Brook	11/2–11/19/88	5.09	111	6.4	2.24	133	12	85
	5/8–5/18/89	5.00	125	3.5	1.93	125	9	100
	5/18–6/9/89	5.74	49	3.3	1.97	69	0	0
	9/27–10/27/89	5.86	42	4.0	2.25	146	6	0
	5/1–5/21/90	5.05	121	4.6	1.83	205	13	100
Buck Creek	11/2–11/13/88	4.66	350	7.7	2.42	350	11	95
	5/8–5/14/89	4.77	149	5.6	2.07	149	6	100
	5/18–5/30/89	5.01	106	5.0	2.31	106	7	48
	9/27–10/17/89	4.84	132	5.6	2.48	139	20	90
	5/1–5/10/90	4.56	201	5.0	2.28	201	9	100
Fly Pond Outlet	11/2–11/19/88	6.70	21	7.8	3.80	21	0	5
	5/8–5/18/89	6.28	25	4.3	3.74	25	0	0
	5/18–6/9/89	6.39	25	4.0	4.80	25	0	0
	9/27–10/27/89	6.70	21	6.4	5.51	21	0	0
	5/1–5/21/90	6.80	20	5.1	4.11	20	0	0
Seventh Lake Inlet	11/2–11/17/88	5.05	220	9.3	3.01	220	15	95
	5/8–5/16/89	4.72	163	6.7	2.44	163	8	95
	5/18–6/9/89	5.18	95	6.0	2.55	105	6	0
	9/27–10/27/89	5.47	64	8.3	2.82	118	6	5
	5/1–5/19/90	4.83	130	7.8	2.36	255	13	95

[a]Daily Al_{im} values were calculated from minimonitor pH measurements using the stream-specific relationship between pH and periodic measurements of Al_{im}.

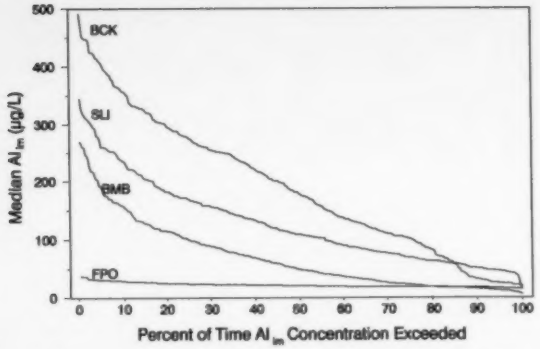

FIG. 2. Percent occurrence of daily median Al_{im} concentrations at the four study streams during the period November 1988 to June 1990. Daily median Al_{im} values were calculated from continuous records of minimonitor pH using stream specific predictive equations. BCK, Buck Creek; SLI, Seventh Lake Inlet; BMB, Bald Mountain Brook; FPO, Fly Pond Outlet.

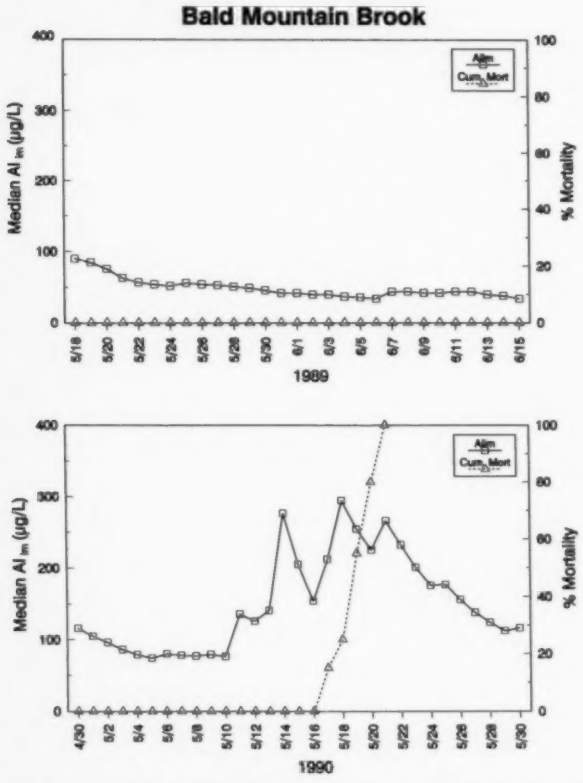

FIG. 3. Cumulative mortality of blacknose dace and calculated median daily Al_{im} during the spring 1989 and 1990 bioassays in Bald Mountain Brook.

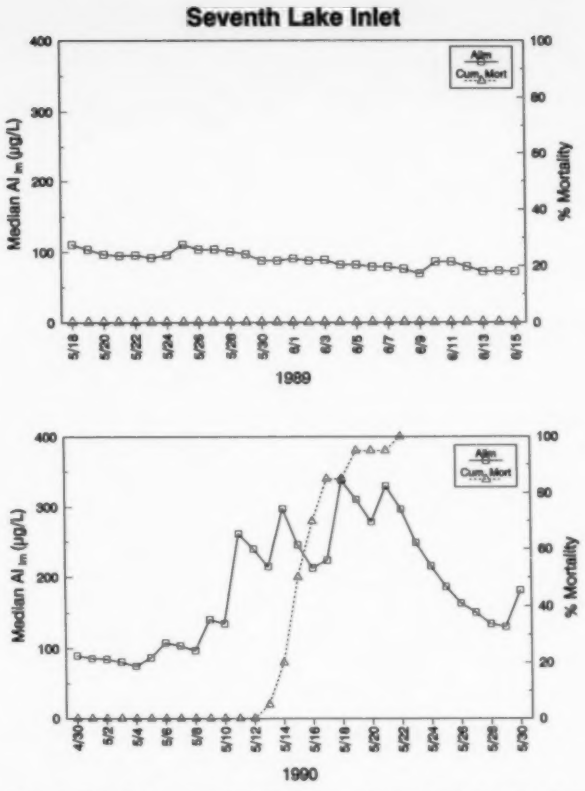

FIG. 4. Cumulative mortality of blacknose dace and calculated median daily Al_{im} during the spring 1989 and 1990 bioassays in Seventh Lake Inlet.

The in situ bioassays consistently demonstrated that the blacknose dace were more sensitive than the brook trout to the episodes of acidic water. Cumulative mortality was greater and occurred sooner in blacknose dace than in brook trout (Fig. 5 and 6). In many cases, all of the blacknose dace died over a short length of time. Brook trout mortality occurred slower and over a longer time period.

Early unexplained mortality of brook trout in the reference stream, Fly Pond Outlet, during the spring 1989 and fall 1989 bioassays may have been caused by handling stress. These bioassays were restarted with new fish in all four study streams. Bioassays were generally considered to be acceptable until the mortality in Fly Pond Outlet exceeded 10%. Because of the variability in water quality among streams, the duration of bioassays varied. Buck Creek exhibited the most toxic water chemistry and therefore the shortest bioassays.

Seventh Lake Inlet was generally more acidic and had higher Al_{im} levels than Bald Mountain Brook both during baseflow and in response to episodes. The bioassay results (Fig. 5 and 6) showed that Bald Mountain Brook was consistently less toxic to blacknose dace than Seventh Lake Inlet. However, Bald Mountain Brook was more toxic to both brook trout and brook trout fry than Seventh Lake Inlet. Although a major epsiode occurred during the spring 1990 bioassay the brook trout fry in Seventh Lake Inlet suffered no mortality whereas the Bald Mountain Brook fish experienced 30% mortality. Seventh Lake Inlet had consistently higher DOC and higher Ca concentrations than Bald Mountain Brook, and this difference may explain the observed bioassay results.

Differences in water chemistry were observed among the four bioassay periods (Table 1), and combining the data allowed for full evaluation of the factors causing fish mortality. DOC, for

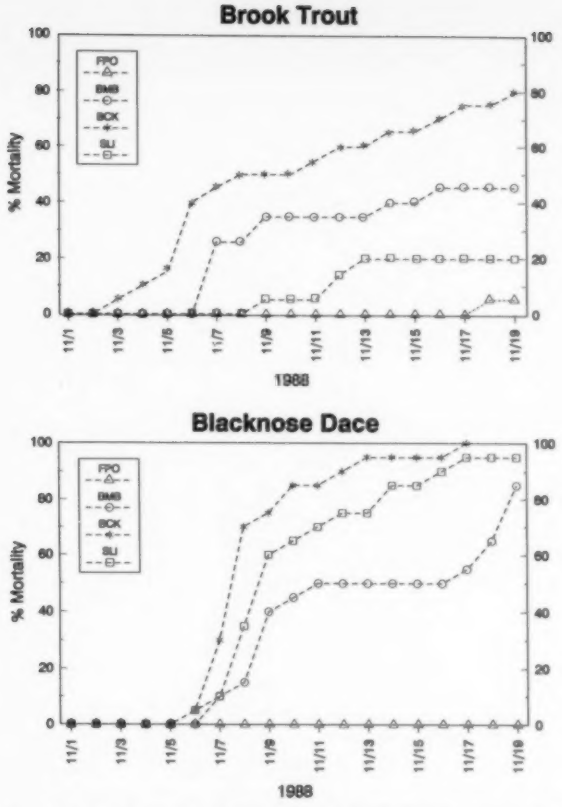

FIG. 5. Cumulative mortality of brook trout and blacknose dace observed during the fall 1988 bioassays. FPO, Fly Pond Outlet; BMB, Bald Mountain Brook; BCK, Buck Creek; SLI, Seventh Lake Inlet.

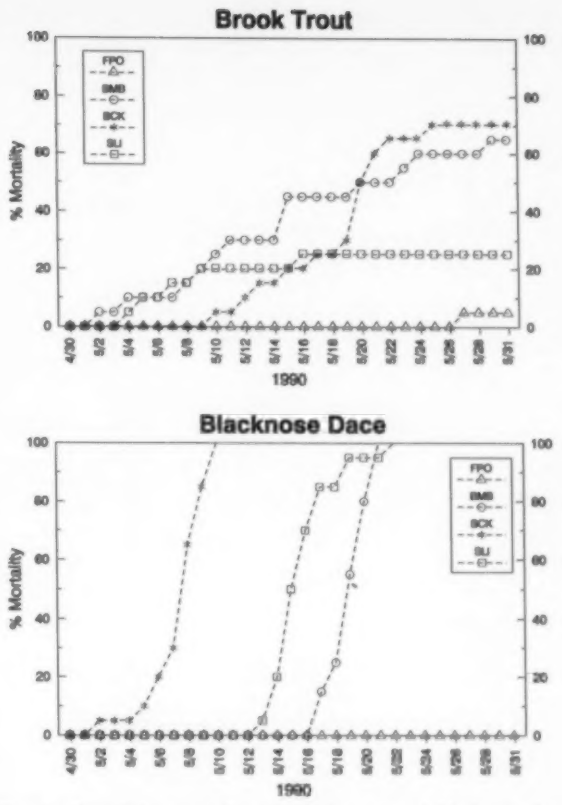

FIG. 6. Cumulative mortality of brook trout and blacknose dace observed during the spring 1990 bioassays. FPO, Fly Pond Outlet; BMB, Bald Mountain Brook; BCK, Buck Creek; SLI, Seventh Lake Inlet.

example, appeared to be somewhat higher in Fly Pond Outlet and Seventh Lake Inlet during the fall bioassays than during the spring bioassays. Two years of data, however, were inadequate to analyze seasonal variation.

During the fall 1989 bioassay, a major episode occurred after 24 d of baseflow conditions (Fig. 7). The episode resulted in negligible mortality in both blacknose dace and brook trout in Bald Mounain Brook and Seventh Lake Inlet, even though high levels of Al_{im} were reached. Other bioassay tests with both species indicated that had this episode occurred at the beginning of the bioassay, significant mortality would have occurred. An early spring 1989 bioassay with blacknose dace (Fig. 7) began with Al_{im} concentrations greater than 100 μg/L and resulted in high mortalities after 6 d. The fall 1989 episode occurred after the fish may have acclimated to the stream or recovered from handling stress. The water quality conditions during this test were typical of those under which fish in a stream would survive. The data from these fall 1989 bioassays showed that blacknose dace in Bald Mountain Brook were able to survive a 6-d episode that resulted in a median Al_{im} concentration of 146 μg/L and median pH of 4.9. Brook trout in Buck Creek experienced only 10% mortality during a 10-d episode that resulted in a median Al_{im} concentration of 199 μg/L, median pH of 4.6, and median DOC of 8.5 mg/L.

A stepwise regression analysis of key variables was conducted to determine which were the best predictors of mortality in the bioassay fish. For blacknose dace the log of median Al_{im} was the best predictor of mortality ($R^2 = 0.75$). Converting the mortality data to probits did not result in better correlations. Figure 8 demonstrates that for blacknose dace, a median Al_{im} concentration of 100 μg/L or less over the entire bioassay period resulted in good survival. The median Al_{im} for the fall 1989 bioassay discussed above was 42 μg/L (Table 1).

The length of time blacknose dace were exposed to elevated Al_{im} concentrations was important and was demonstrated during several episodes. Mortality of blacknose dace during the spring 1990 bioassay did not occur until several days after the start of the episode (Fig. 3 and 4). If an episode occurred for over 6 d, high mortality occurred in the blacknose dace bioassays (Fig. 8). CONCDUR was also identified as a key variable in the stepwise analysis. The data indicate that median Al_{im} concentrations greater than 100 μg/L and episodic peaks of over 6 d resulted in mortality to blacknose dace. When the predicted Al_{im} concentrations for each stream (Fig. 2) are examined, it is evident why blacknose dace do not survive well in Buck Creek, Seventh Lake Inlet, and Bald Mountain Brook. The percentage of time the predicted Al_{im} was greater than 100 μg/L was approximately 76% for Buck Creek, 57% for Seventh Lake Inlet, and 25% for Bald Mountain Brook.

When a stepwise regression analysis of key variables was conducted on the brook trout bioassay data, CONCDUR was the best predictor of mortality and resulted in the best one-

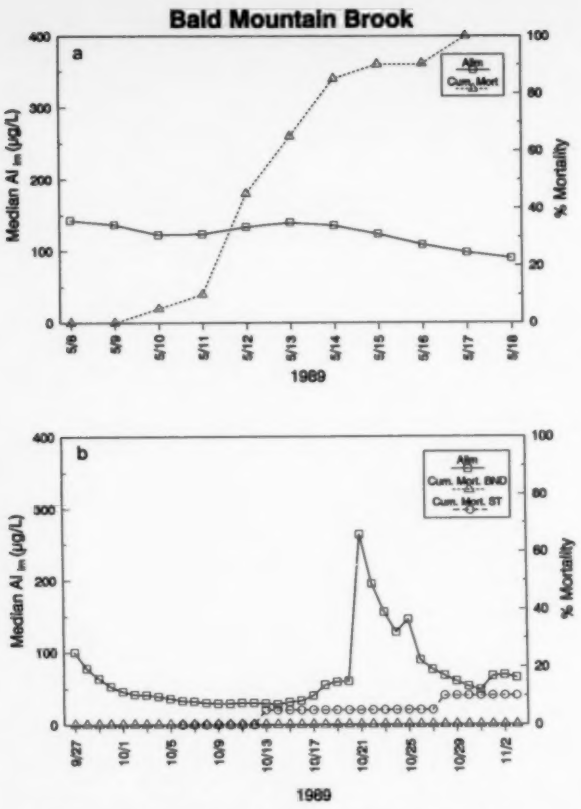

FIG. 7. Cumulative mortality and calculated median daily Al_{im} of (a) blacknose dace during the spring 1989 bioassays and (b) blacknose dace (BND) and brook trout (ST) during the fall 1989 bioassays in Bald Mountain Brook.

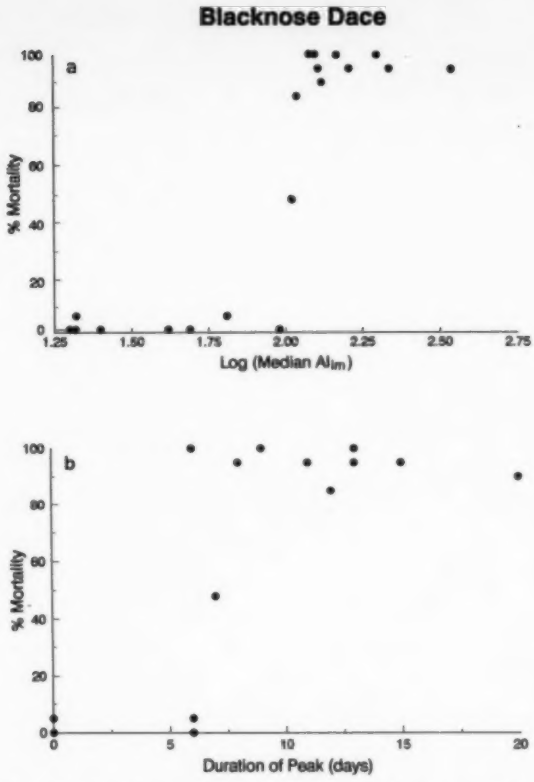

FIG. 8. Cumulative mortality of blacknose dace in relation to (a) the log of median Al_{im} during the bioassay and (b) the duration of the episode. Each data point represents a separate bioassay in one of the four streams.

variable model (R^2 = 0.44). As with the blacknose dace, converting the mortality data to probits did not improve the R^2 term. The second most important variable of those tested proved to be DOC, and when both CONCDUR and DOC were used to predict mortality the R^2 term increased to 0.61. Figure 9 shows the predicted response surface if these two variables are used to predict brook trout mortality. Increases in DOC result in decreased predicted mortality to brook trout. The best two-variable predictive equation for brook trout was therefore as follows:

$$\text{Percent mortality} = 49.7 + 0.01(\text{CONCDUR}) - 6.46(\text{DOC}).$$

The brook trout bioassay data were more variable than the blacknose dace data with mortalities occurring over a greater range of water chemistry. Although cumulative mortality figures similar to Fig. 8 were developed for brook trout, they included a number of unexplainable outliers. However, it is evident that brook trout can withstand a slightly higher median Al_{im} concentration for a longer duration than blacknose dace.

The hatchery-reared brook trout fry were treated as a separate group of fish, and the data from these bioassays were also analyzed using the maximum R^2 procedure. The log of the median calcium concentration proved to be the best single variable, but only produced an R^2 term of 0.33. The best two-variable model of the brook trout fry data included CONCDUR and the log of median Al_{im} and resulted in an R^2 of 0.71. The brook trout fry data therefore appear to be consistent with the findings from the wild brook trout and the blacknose dace bioassays.

Discussion

The results of the bioassay tests from this study confirm previous findings that blacknose dace are more sensitive to acidic, high-Al_{im} waters than are brook trout. Johnson et al. (1987) presented similar bioassay results, and Kretser et al. (1989) presented survey data of 1469 Adirondack waters which showed that blacknose dace occurred at a minimum pH of 5.59 and brook trout at a minimum of 4.64. Schofield and Driscoll (1987) listed blacknose dace as a species sensitive to acidification and brook trout as indeterminate in its sensitivity.

Fish population studies were also a part of this project (Kretser et al. 1991). The results of those studies are in general agreement with the bioassay data and show Buck Creek to be the most toxic of the four study streams. Blacknose dace stocked in the three most acidic streams suffered poor survival, while greater numbers of brook trout were able to survive. In some cases, brook trout and a few blacknose dace in the stream were able to survive, while in situ bioassay fish died.This may be due to several factors including stress on caged fish resulting from handling and confinement. As discussed below, the bioas-

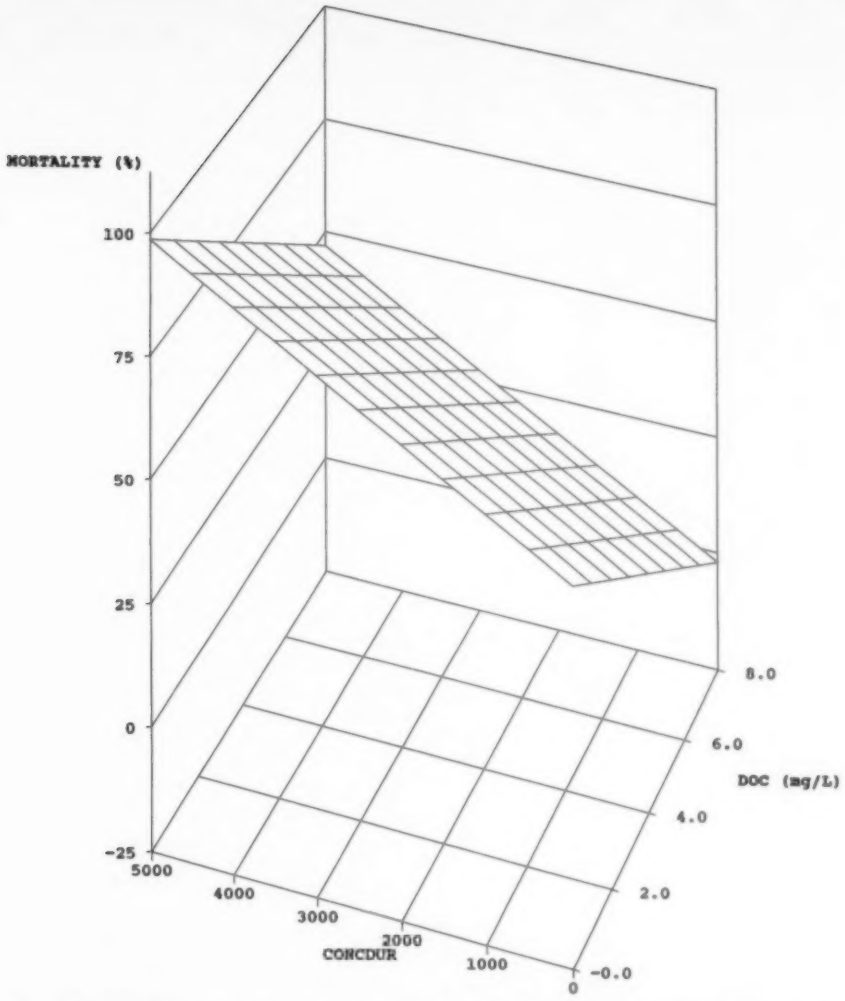

FIG. 9. Predicted mortality of brook trout in terms of DOC and CONCDUR. CONCDUR was defined as the median Al_{im} concentration during an episode (period of daily median $Al_{im} > 100$ μg/L) multiplied by the duration of the episode in days. The equation for the response surface is Percent mortality = 49.7 + 0.01(CONCDUR) − 6.46(DOC).

say fish may not have been acclimated to baseflow conditions for as long a period as the stream fish, resulting in higher mortality in the bioassay fish. There is also the possiblity that wild fish may encounter small areas under banks with slightly better water quality.

The bioassay results showed that after the test fish were in the stream for 15–24 d, they could better withstand acidic episodes. They were able to survive several days of water quality conditions that would have caused fish mortality if they had occurred early in the bioassay period. Although the bioassay fish may not have survived a major episode of long duration, they appeared to become at least partially acclimated to the water quality and/or to recover from the initial stress of being handled and caged. This acclimation was observed in both brook trout and blacknose dace. Mount et al. (1990) also observed similar acclimation to baseflow conditions in laboratory experiments with brook trout. Fish that they exposed for 18 d to baseline conditions (pH 5.21 and Al_{im} concentration of 21 μg/L survived a 6-d acidic episode (pH 4.59 and Al_{im} of 244 μg/L), while fish without prior acclimation that had been reared at pH 6.5 suffered over 50% mortality when exposed to the same acidic, high-aluminum episode.

DOC was an important variable in the brook trout bioassays. Fish mortality was less in streams with higher DOC levels, even though Al_{im} levels measured were the same. The higher DOC and calcium levels in Seventh Lake Inlet may have allowed better brook trout survival there than in Bald Mountain Brook. This was the case in spite of the fact that Al_{im} concentrations appeared in most cases higher in Seventh Lake Inlet. The blacknose dace in these streams may either have been more sensitive to the Al_{im} concentrations than the brook trout or were not protected by the higher DOC and calcium levels.

The finding that in situ DOC concentrations were positively correlated with brook trout survival agrees with laboratory data.

TABLE 2. Number of in situ bioassays where observed mortality was correctly predicted (within ± 10%) by predictive models developed in other studies. Brook trout feeding fry bioassays were conducted during spring 1989 and spring 1990. Brook trout yearling and blacknose dace bioassays were conducted during spring and fall 1989 and spring 1990. The predictive models assume stable water chemistry conditions, and the field data used to test the models were 10-d periods of relatively stable conditions. The number of bioassays in which the model predicted more mortality than was observed is given in parentheses. NA = not applicable.

	Johnson et al. (1987)	Parkhurst (1987) 1	Parkhurst (1987) 2	Ingersoll et al. (1990)	Parkhurst et al. (1990)	Baker et al. (1990)
Brook trout feeding fry	2 of 8 (6)	0 of 8 (8)	2 of 8 (6)	5 of 8 (1)	3 of 5 (5)	5 of 8 (1)
Brook trout yearlings	7 of 12 (1)	NA	NA	NA	NA	NA
Blacknose dace adults	2 of 12 (10)	NA	NA	NA	NA	NA

Parkhurst et al. (1990) conducted laboratory bioassays that demonstrated that brook trout mortality was reduced at higher DOC concentrations. The predicted response surface based on our field studies (Fig. 9) is similar to the values they presented, showing the highest brook trout survival at low aluminum and high DOC concentrations. Parkhurst et al. (1990) also found that the best predictive equation of brook trout survival for their data included Al_{im}, DOC, and pH. In earlier field studies, Parkhurst (1987) had not observed a beneficial effect of higher DOC concentrations, possibly because in those studies, DOC was negatively correlated with pH.

It is interesting to note that DOC is generally thought to bind the toxic Al_{im} and convert it to Al_{om}. Therefore, measurements of Al_{im} should already include the benefit derived from DOC. The interpretation of the DOC and Al_{im} interactions may be complicated by the analytical method used for Al_{im} measurement. Recent research at the University of Wyoming has shown that using an ion exchange resin column (Driscoll 1984) to separate the organic and inorganic fractions of monomeric aluminum (as was done in our study) may lead to an overestimation of Al_{im} (Bergman 1992). Weak organic acids may be able to bind with Al_{im} and reduce its bioavailability, but may not be strong enough to remain bound to the Al_{im} in an ion exchange resin column. Consequently the measured Al_{im} value may include some of the Al_{om}. In higher DOC waters, such as Seventh Lake Inlet, much of what was measured as Al_{im} may in fact be Al_{om} and not be toxic to the fish. A better method of analyzing for the biologically available fraction of aluminum is needed.

Our bioassay and water chemistry monitoring results indicated that Al_{im} was the primary parameter that caused fish mortality during acidic episodes. Baker and Schofield (1982), Johnson et al. (1987), Parkhurst et al. (1990), and others also identified Al_{im} as a primary determinant in the survival of fish exposed to acid waters. These researchers also developed models to predict fish survival under various water quality conditions. These models, however, did not generally predict what we observed in our bioassays (Table 2). The observed mortality was correctly predicted in greater than 50% of the bioassays only when we used models developed by Ingersoll et al. (1990) or Baker et al. (1990) for brook trout feeding fry or the model developed by Johnson et al. (1987) for brook trout yearlings.

The reasons for the relatively poor agreement between the predicted mortality and our observed bioassay data may be attributed to a number of factors. These may include the use of other methods to measure Al_{im}, variation in fish strain or age, variation in fish handling, the fact that test fish may not have been acclimated to low-conductivity water quality conditions, and/or the fact that the brook trout feeding fry used in the study described here were of hatchery origin. Each predictive model had a set of experimental conditions that may have been different from the bioassays we conducted. Although these models are useful in gaining a better understanding of how variations in water chemistry affect fish survival, they do not fully characterize the variables occurring in the stream environment. The occurrence of acidic episodes is the primary factor that is not taken into account in any of these predictive models.

The variable CONCDUR was developed to incorporate acidic episodes into predictive models. CONCDUR was found to be an important variable correlated with mortality of bioassay fish. Brook trout were able to survive episodes for a longer duration than blacknose dace, and both appeared to survive longer than expected based on research by Gagen and Sharpe (1987). They found that in Linn Run, Pennsylvania, brook trout exposed to over 200 μg Al/L for durations greater than 1 d suffered mortality. In our streams, exposures of longer duration were necessary to cause brook trout mortality. DOC may not have been as important in Linn Run as in our study streams. As well, the unacclimated hatchery brook trout they used may have been more sensitive to the test conditions than were the wild brook trout used in this study.

The in situ bioassay tests reported here documented that our study streams were toxic to fish at certain times and that episodes of toxic water chemistry did occur. During these episodes, pH levels were frequently less than 5.0 and Al_{im} levels greater than 100 μg/L. The continuous water chemistry monitoring data showed that episodes often lasted for many days and in some cases several weeks (spring 1990 bioassays). Episodes of sufficient duration resulted in mortality in the bioassay fish.

Extended seasonal periods of poor water quality resulting in fish mortality may be more important in our study streams than short acidic episodes. These periods can result from a number of snowmelt or precipitation episodes occurring one after another before the stream returns to baseflow conditions. High flows and associated high Al_{im} levels for over 6 d resulted in blacknose dace mortality. Episodes of longer duration were necessary to cause significant brook trout mortality. Although duration of acidic episodes appears to be a critical characteristic, the term "chronically acidified" does not truly apply to

these streams. During periods of baseflow conditions, which in our streams occur about half of the time, each stream provides acceptable water quality for fish survival. Critical conditions for fish survival occur when episodes of toxic water quality continue for many days. Fish are able to survive episodes of short duration.

It has been hypothesized that wild fish may move to refuge areas of better water quality during episodes whereas caged bioassay fish are confined to one location. Large refuge areas where fish can congregate to withstand acidic episodes did not appear to be an important characteristic of our Adirondack study streams. A small refuge site in Buck Creek was located, and Seventh Lake was a potential downstream refuge area for both Buck Creek and Seventh Lake Inlet. However, they did not play an important role in terms of fish survival during episodes (D.W. Bath, Adirondack Lakes Survey Corporation, Ray Brook, NY, unpublished data). The primary factor affecting fish populations in our study streams appeared to be the occurrence of episodes of poor water quality. Fish were not observed to actively seek refuge sites during episodes and then disperse throughout the stream following the episodes.

Carline et al. (1992) reported that in Pennsylvania, greater than 3000 km of trout streams have been adversely influenced by acidification. Water chemistry survey data for Adirondack headwater streams are limited, but studies by Colquhoun et al. (1983, 1984) showed that many streams are fishless, particularly those in the southwestern Adirondacks, and have spring pH levels less than 5.0. The periodic toxicity of certain Adirondack streams due to episodic acidification is likely the reason why they either are fishless or support limited fish populations. One month or less of acidic water quality could either reduce or eliminate fish populations. The impact may be evident for years in streams that have steep gradients or barriers that block fish from moving back upstream into otherwise suitable habitat. Steep gradients are common in Adirondack headwater streams and may explain the occurrence of some fishless waters.

As was noted by Johnson et al. (1987), we found that the number of waters that are acidic is greatest during spring snowmelt and major runoff events. Toxic water conditions that occur during such episodes cause fish mortality and reductions in stream fish populations. Future research on this subject needs to incorporate at least three key factors: a better method for measuring the biologically relevant Al_{im}, consideration of the fact that fish do become acclimated to low-conductivity water quality, and consideration of the fact that episodes of acidic water quality play a key role in determining the survival of stream fish populations.

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Feeding, Growth, and Survival of Atlantic Cod (*Gadus morhua*) Larvae Reared in Replicate Plastic Enclosures

Håkon Otterå

Institute of Marine Research, Department of Aquaculture, P.O. Box 1870, Nordnes, N-5024 Bergen, Norway

Otterå, H. 1993. Feeding, growth, and survival of Atlantic cod (*Gadus morhua*) larvae reared in replicate plastic enclosures. Can. J. Fish. Aquat. Sci. 50: 913–924.

Feeding, growth, and survival of Atlantic cod (*Gadus morhua*) larvae were examined during their first 2 mo of life. The experiment was carried out in eight plastic enclosures of 10 m^3 volume each. Larval cod, at an initial stocking density of 10 larvae/L, were fed rotifers (*Brachionus plicatilis*) and natural zooplankton collected from the sea. The enclosures replicated well with regard to hydrography, feeding conditions, and larval growth and survival. Larval mortality was low during the first month, with a mean mortality rate (*Z*) of 0.02/d. Growth, however, was very slow and mortality increased significantly after about 4 wk, possibly due to starvation. *Brachionus plicatilis* dominated the gut content during the first 3–4 wk. The zooplankton concentration averaged 15–80 rotifers/L during the first 2 wk, which seemed to be insufficient under the present rearing conditions. Most of the larvae were distributed in the upper parts of the rearing enclosures, while rotifers were distributed deeper. This, together with the high larval density, inadequate rotifer enrichment, and low temperature probably amplified the unfavourable feeding conditions.

L'alimentation, la croissance et la survie des larves de morue franche (*Gadus morhua*) ont été étudiées au cours des 2 premiers mois de leur vie. L'expérience a été réalisée dans huit enceintes de plastique de 10 m^3 chacune. Les larves de morue, dont la densité initiale était de 10 larves/L, étaient alimentées de rotifères (*Brachionus plicatilis*) et de zooplancton naturel provenant de la mer. Les enceintes remplissaient bien leur fonction en ce qui a trait aux conditions hydrographiques, aux conditions d'alimentation et à la croissance et à la survie des larves. La mortalité larvaire a été faible au cours du premier mois, le taux de mortalité moyen (*Z*) étant de 0,02/jour. Toutefois, la croissance a été très lente et la mortalité a augmenté substantiellement après environ 4 sem, probablement à cause de la privation de nourriture. *Brachionus plicatilis* a dominé dans le contenu intestinal au cours de 3 ou 4 premières semaines. La concentration de zooplancton moyenne était de 15–80 rotifères/L au cours des 2 premières semaines, ce qui a semblé insuffisant dans les conditions d'élevage utilisées. La plupart des larves se trouvaient dans la partie supérieure des enceintes d'élevage et les rotifères vivaient plus profondément. Ce facteur, combinée à une densité larvaire élevée, à un enrichissement en rotifères inapproprié et à un température faible, ont probablement amplifié les conditions défavorables pour l'alimentation.

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Studies of growth rates and their variability and possible interactions with mortality rates in early life stages of fishes are a useful way to examine recruitment potential (Houde 1987). Results obtained from field and laboratory studies of larval and juvenile fish ecology are, however, often inconsistent, especially with respect to the relationship between prey densities and larval growth and survival. Laboratory experiments usually indicate higher abundance requirements for prey compared with those observed in the field (Hunter 1981). This may be due to either inadequate zooplankton sampling in the field, as suggested by Frank (1988), patchy distribution of zooplankton in the sea (Hunter 1981; Fortier and Leggett 1984), or differences in small-scale turbulence (Rothschild and Osborne 1988; Sundby and Fossum 1990). Others have raised doubts about the validity of results obtained from laboratory studies, under conditions which deviate dramatically from those natural for the larvae (Gamble 1985; de Lafontaine and Leggett 1987b).

Studies performed in mesocosms or enclosures, where conditions are relatively controlled but not far from natural have proved to be valuable in bridging the conflicting results from laboratory and field (Øiestad 1982; Gamble 1985; Houde 1987). Walde and Davies (1984) listed four characteristics of an ideal enclosure: they should (1) replicate well, (2) give good reproducibility of the natural environment, (3) establish a representative community, and (4) have predator–prey relationships at natural densities and ratios.

Several investigations with various objectives and types of enclosure have been carried out with fish larvae (reviewed by Gamble 1985). Since 1983, hundreds of thousands of Atlantic cod (*Gadus morhua*) juveniles have been produced using large (>60 000 m^3) enclosures (Øiestad et al. 1985b; Blom et al. 1991). This has facilitated production of cod juveniles for aquaculture purposes in addition to revealing fundamental ecological processes. Such large enclosures probably meet the last three requirements of an ideal enclosure, but they preclude use of replicates. An assessment of the variability among groups equally treated is crucial both in experimental ecological designs (Hurlbert 1984) and when evaluating a production method for aquaculture purposes.

In this study, I examined aspects of the feeding ecology, growth, and survival of cod larvae and, in particular, how well these factors would replicate under similar rearing conditions. An experimental design of eight equally treated enclosures

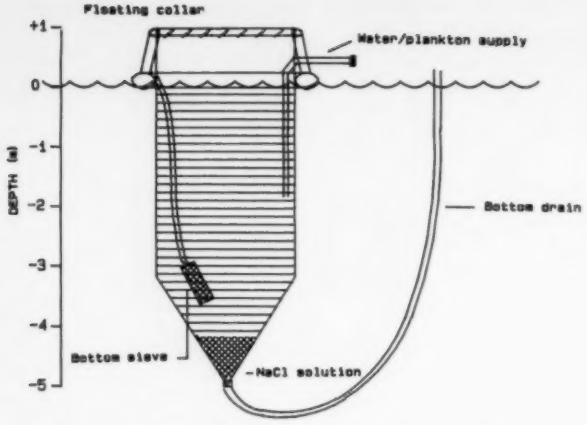

FIG. 1. Cross-section of experimental enclosure.

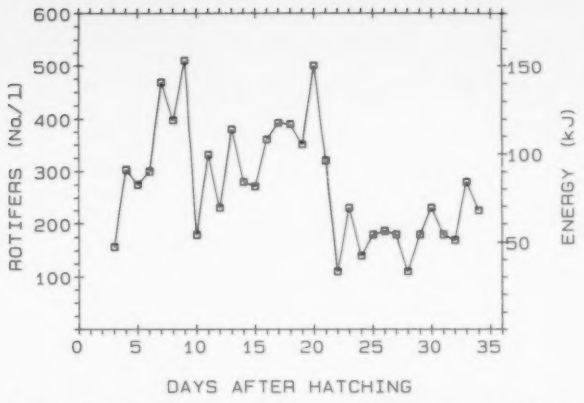

FIG. 2. Quantity of rotifers (*Brachionus plicatilis*) supplied to the experimental enclosures. Values are given in numbers per litre of enclosure volume; total energy content is estimated from Theilacker and Kimball (1984).

(10 m^3), and where larval food was collected from the sea or cultured in the laboratory, was therefore set up.

Materials and Methods

The experiment was carried out at Austevoll Aquaculture Research Station, Institute of Marine Research, Norway, during the spring of 1984.

Enclosures

The enclosures were made of light grey polyethylene, and each enclosure (diameter × depth = 2 × 5 m) contained approximately 10 m^3 of water. They were filled with filtered seawater (10 μm) from 50 m depth 3 d before the larvae were released. The experimental enclosures were covered by a net (4-mm mesh size), reducing the ambient light level to about 76%. A water exchange rate of 7 L/min, which corresponds to 100% renewal per day, was maintained throughout the experiment. Inflow was supplied into the surface of the enclosures and drained through a bottom sieve (Fig. 1). Eight enclosures, bounded together in two rows, were used in the experiment.

Larvae

Fertilized cod eggs were collected from a plastic pen where 56 female and 68 male cod spawned naturally (Huse and Jensen 1983). The eggs were incubated in 100-L plastic cylinders at a temperature of 4–8°C.

TABLE 1. Enrichment feed for the rotifers.

	% (weight)
Fish meal	55.0
Peptonal	25.0
Cod liver oil	10.0
Vitamine premix[a]	4.0
Premix[b]	6.0

[a]Described by Gatesoupe and Luquet (1981).

[b]Choline chloride, 3.0%; DL-methionine, 1.5%; $CaHPO_4$, 0.8%; $FeSO_4 \cdot 7\ H_2O$, 0.2%; Carophyll red, 0.5%.

On March 16, 3 d after 50% hatching (defined as day 0), cod larvae were randomly transferred in roughly equal numbers to the eight experimental enclosures. An estimated total number of 834 000 larvae (SD = 111 000) gave an initial density of ≈10/L.

Husbandry and Food Production

Sediment and dead larvae were removed from the enclosures twice a week through the bottom drain (Fig. 1). Twenty litres of saturated NaCl solution was then added to the bottom of each enclosure and remained until the next extraction to prevent live larvae from actively swimming near the bottom.

The larvae were offered rotifers (*Brachionus plicatilis*) twice daily from day 3 to 22 and once daily from day 23 to 34 and natural zooplankton from day 3 to the end of the experiment. Natural plankton was collected from 5 m depth by pumping seawater (7 m^3/min) through a double plankton net (Huse et al. 1983). The inner net had a mesh size of 250 μm and retained the larger zooplankton which were unsuitable as larval food. The outer (90 μm) concentrated plankton of the desired size. As the larvae grew, coarser nets were used (500/120 μm). Plankton and water from the outer net were led through a pipe into a collecting cage and further pumped into the enclosures.

The rotifers were cultured in the laboratory according to methods described by Gatesoupe and Luquet (1981) and fed with the algae *Nannochloris atomus*. The rotifer culture was enriched with fish meal (Table 1) for about 10 h before being fed to the larvae at about 10:00 and 15:00 every day. The variable amount of rotifers supplied to the larvae (Fig. 2) reflects the problems of maintaining a high and stable density in the production facilities. Each enclosure was supplied with approximately the same amount of plankton and rotifers.

The experiment ended when the enclosures were emptied 56–70 d after hatching.

Sampling and Analysis

Surface water temperature and salinity were measured daily in three of the enclosures. Additional samples taken at 1-m intervals indicated homogenous water masses. Oxygen concentration was measured weekly throughout the water column using an YSI oxygen probe.

Zooplankton and larvae were sampled during daytime three times a week from day 4 by quickly hauling a 4.5-m-long, 6-cm-diameter plankton net (mesh size 90 μm) from the bottom of the enclosure to the surface. Three replicates were taken from each enclosure. The number of larvae was recorded and the samples were fixed in 4% hexamine neutralized formaldehyde. On day 8, additional evening samples were also taken.

Two of the enclosures were sampled for larvae and zooplankton six times over a 24-h period on days 22–23. Triplicate 5-L water samples were taken at the surface, 2 m depth, and just above the bottom of the enclosures using Niskin bottles. Depth and time effects of larval numbers and gut content were tested by a Model 1, two-way ANOVA on each enclosure separately. The tests were done on the square root of the number of larvae per sample and the square root of the numbers of prey + 0.5 per gut (Sokal and Rohlf 1981).

Zooplakton were examined under a stereomicroscope and classified to the major taxonomic groups. Differences in plankton populations between the enclosures were tested with a one-way ANOVA on ranked values calculated for each sampling date. In addition to zooplankton, large phytoplankton like *Coscinodiscus* spp., *Ceratium* spp., *Halosphaera* spp., and *Peridinium*/*Paraperidinium* spp. were counted from the samples.

Twenty larvae from each enclosure were examined on each sampling date, 10 gutted and 10 ungutted. Standard length (SL), myotome height at anus, and gut development of the larvae were recorded according to Fossum (1986). The larvae were dried (24 h; 60°C) and weighed with an electrobalance (±1 μg). The gut content was classified to major taxonomic groups and counted. Each food item was also classified as digested, partly digested, or not digested. Differences in gut content among the enclosures were tested by one-way ANOVA on ranked values.

Prey selection was calculated using Chesson's α:

$$\sum_{i=1}^{m} \alpha_i = 1; \alpha_i = r_i n_i \left(\sum_{j=1}^{m} r_j n_j^{-1} \right)^{-1}; i = 1, \ldots, m$$

where n_i is the proportion (in numbers) of prey$_i$ in the enclosure and r_i is the proportion of the same prey in the larval gut (Chesson 1978). Chesson's α was calculated for rotifers, nauplii, and copepods, the most numerous prey found in the guts.

The following equation obtained from mean weights of larvae of age 4–31 d describes the relationship between gutted and whole larvae and was used for pooling gutted and whole weights:

$$W_{\text{whole; μg}} = 1.40 \times W_{\text{gutted; μg}} - 11.45;$$
$$R^2_{\text{corr.}} = 0.96; N = 13.$$

Exponential growth was estimated by linear regression of ln-transformed dry weights and SLs on age. Only weights from day 10 were used in the regression because of the decrease during yolk absorption. Transformation to natural logarithms normalized the values but did not induce homoscedasticity in either lengths or dry weights. To compensate for the increasing variance with age, the fits were weighted by the reciprocal of the variance per day and enclosure.

Specific growth rates for single larvae were calculated as $100 \times (e^g - 1)$. The daily instantaneous growth rate g was calculated as

$$(\ln W_t - \ln W_4) / (t - 4)$$

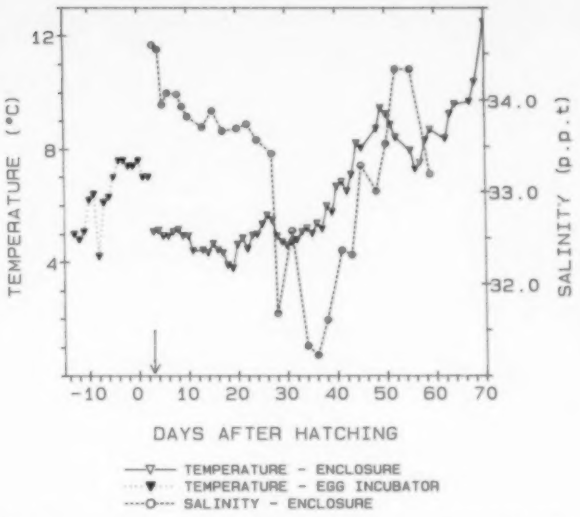

FIG. 3. Temperature in the egg incubators and experimental enclosures, and salinity in the enclosures during the experiment. The arrow indicates the day of release into the enclosures.

where W_t is dry weight (whole larvae) on day t and W_4 is mean dry weight on day 4, which was the first day of sampling.

The number of larvae in each enclosure was estimated from the mean number of larvae in the three samples, multiplied by the ratio of enclosure volume versus sampling volume. At the end of the experiment, all fry from the enclosures were collected with a dip-net, fixed, and SL was measured. Dry weight was measured from a subsample, and the following weight–length relationship was used in calculating growth rates at the end of the experiment:

$$\ln (W_{\text{mg}}) = 0.34 \ln (\text{SL}_{\text{mm}})^2 + 1.43 \times \ln (\text{SL}_{\text{mm}}) - 4.93;$$
$$R^2_{\text{corr.}} = 0.98; N = 1090.$$

Results

Hydrography

Surface temperature in the enclosures was quite stable, about 5°C, during the first 4 wk, thereafter increasing quickly to about 9°C on day 50 (Fig. 3). Salinity was above 33.5 during the first 4 wk but dropped to about 31 between days 30 and 40 (Fig. 3). The observed fluctuations in temperature and salinity reflected those of the surrounding waters. Oxygen saturation varied between 93 and 118%.

Plankton

Coscinodiscus spp. were the most numerous phytoplankton with densities of 24–287/L from day 10 to 31. Smaller chain building diatoms were relatively uncommon.

Brachionus plicatilis was the most abundant zooplankton during the first 4 wk, decreasing from a mean of about 70/L the day after release of the larvae to almost zero on day 38 (Fig. 4). The rotifer density reported in Fig. 4 is the estimated density at the time of sampling and cannot be directly compared with the estimated daily supply reported in Fig. 2 due to grazing effects.

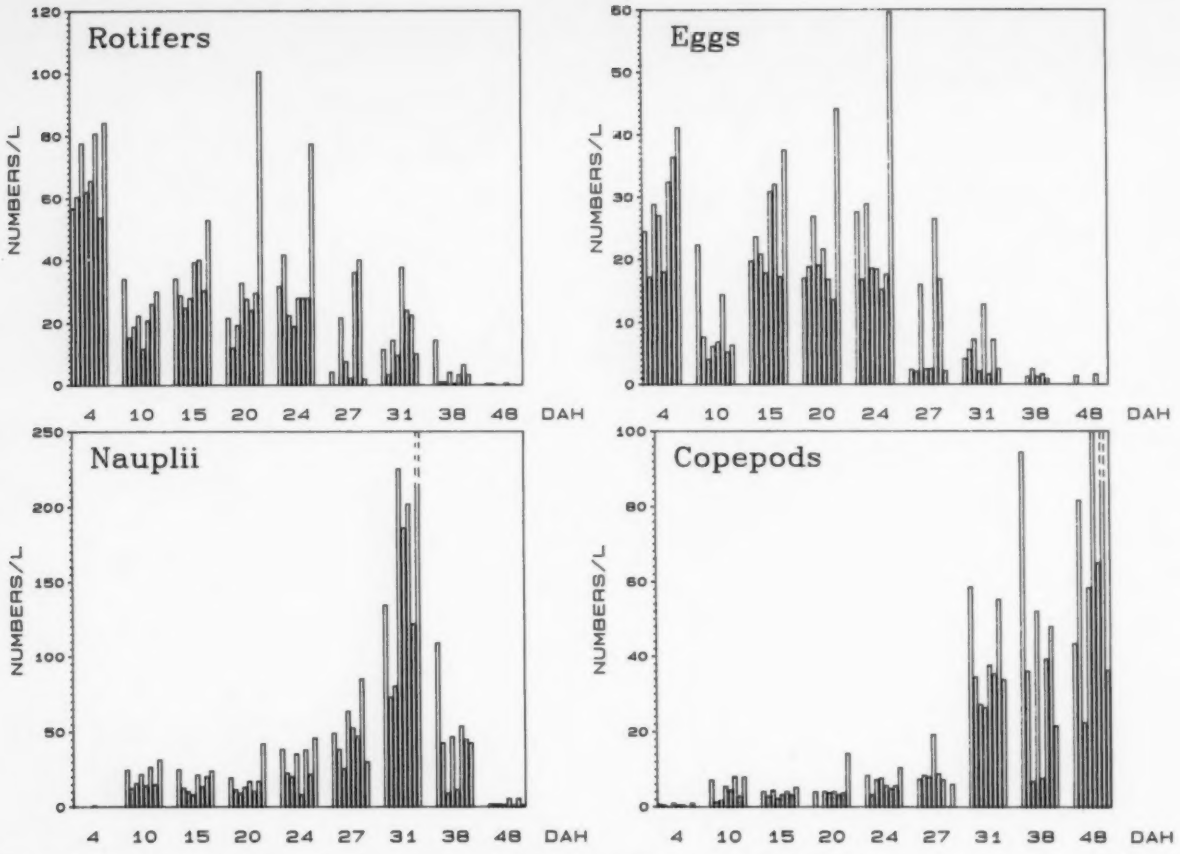

FIG. 4. Density of the most important larval prey as a function of larval age (DAH = days after hatching). Mean values for enclosures 1–8 are given from left to right. The bars representing nauplii in enclosure 8 on day 31 and copepods in enclosure 7 on day 48 have been truncated; the actual numbers were 402 and 379/L, respectively.

The mean number of copepod nauplii increased from about 20/L on day 10 to about 50/L on day 27 and 170/L on day 31. The mean density of copepods was ≤20/L until day 27, increased to about 40/L on day 31 and to 80/L on day 48 (mainly the genera *Pseudocalanus*/*Paracalanus* and *Acartia* and the harpacticoid *Tisbe*). Although Fig. 4 indicates differences in plankton numbers between the enclosures, there were no significant differences in the abundance of rotifers, eggs, or copepods between the enclosures ($P > 0.1$). However, there was a significant difference ($P < 0.01$) for nauplii, with enclosures 1 and 8 having the highest averages.

Feeding

On the first day after release, about 50% of the examined larvae had food in their guts, and from day 15, almost all the examined larvae had food in their guts (Fig. 5). A mean of 1–5 organisms per gut was observed until day 10, rising to between 6 and 24 prey items between days 13 and 24 (Fig. 6). Rotifers and rotifer eggs dominated the gut content until day 24. Copepod nauplii constituted a minor part of the gut content during the first period, but from day 27 to day 41, they were the dominant food item. Copepods were nearly absent in the larval guts until day 41. Other organisms (phytoplankton, larvae of bivalves, gastropods, polychaetes, etc.) were only found occasionally. There were no significant differences in gut content between the enclosures ($P > 0.3$).

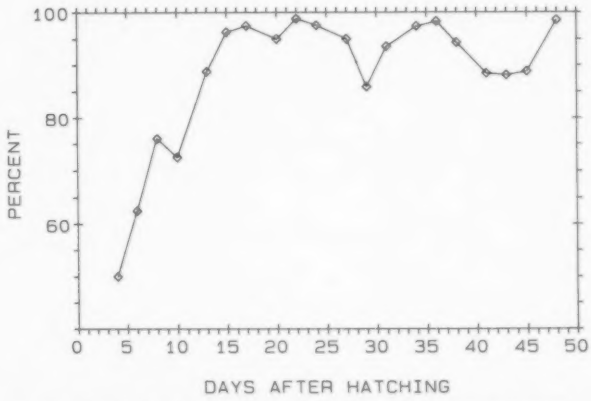

FIG. 5. Feeding incidence of larvae (mean of larvae from all experimental enclosures).

In terms of dry weight, the gut content increased from about 0.3 μg/gut (0.8% of larval weight) on day 4 to a maximum of

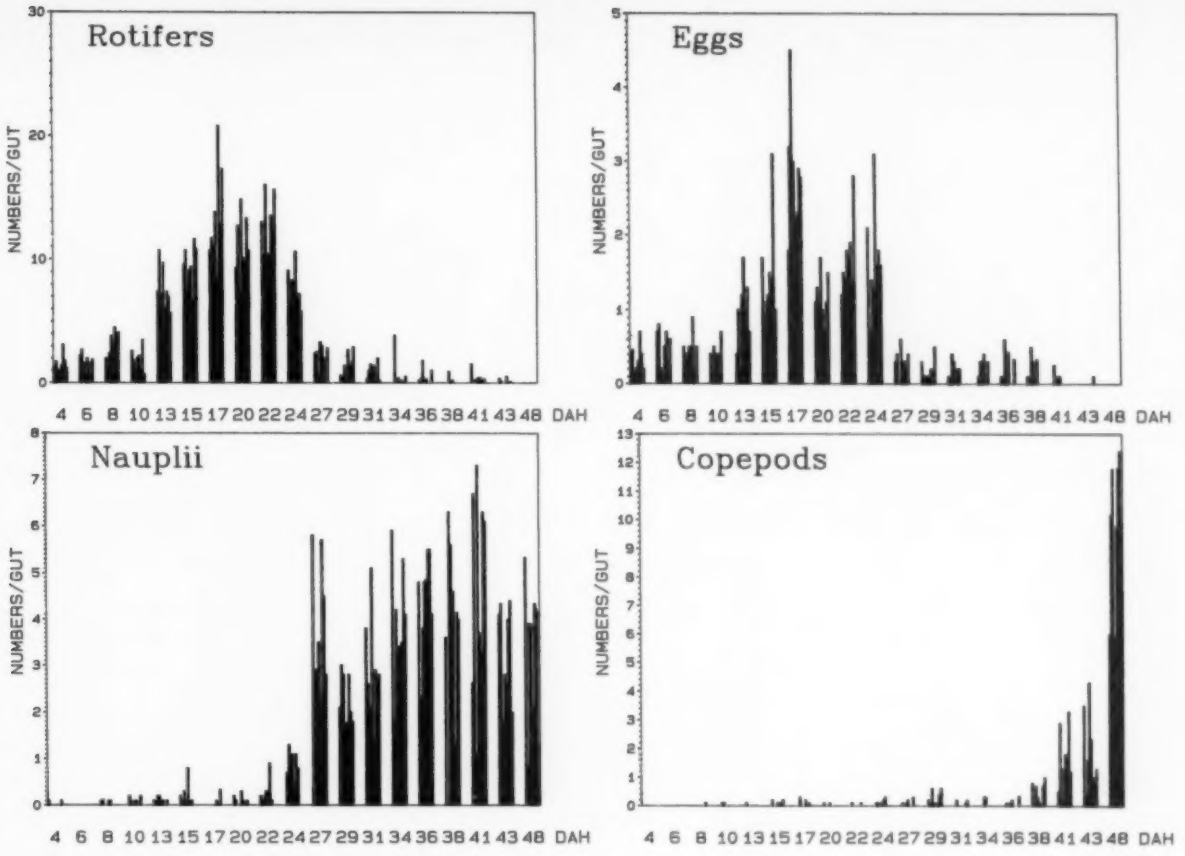

FIG. 6. Numbers of the most important prey per larval gut, as a function of larval age (DAH = days after hatching). Mean values for enclosures 1–8 are given from left to right.

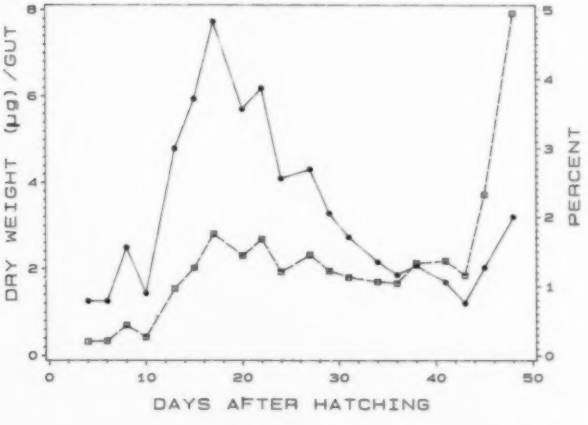

FIG. 7. Average gut content from all enclosures expressed as dry weight per larval gut (squares) and percent of larval dry weight (circles). Dry weight of rotifers was set to 0.20 µg (Theilacker and Kimball 1984), of copepod nauplii to $4 \times TL^{1.65}$ (Øiestad 1983), and of copepods to $18.16 \times L^{2.39}$ (Robertson 1968).

about 2.8 µg/gut (4.8% of larval weight) on day 17 (Fig. 7). From day 17 to day 43, the gut content weight decreased slightly, but rapidly relative to larval weight. The gut content increased from day 45, when copepods had become a major food item in the guts.

Chesson's α revealed a preference for rotifers until day 24 (Fig. 8). On days 27–38, nauplii were the most preferred food, while copepods and nauplii were selected in almost equal amounts on day 48.

Day and Night Sampling

Visual observations and the results from the day and night station (enclosures 4 and 6) revealed significant depth differences in the larval distribution ($P < 0.001$). Most of the larvae were distributed in the upper part of the enclosures (Fig. 9a). No time effect was observed during the sampling period ($P > 0.05$), which suggests that vertical migrations were absent. There was a pattern of higher weights in night-caught larvae than in day-caught larvae (although this was only significant in enclosure 6; T-test; $P < 0.01$). The larval weight also decreased with sampling depth in enclosure 6 (ANOVA; $P < 0.01$) but not in enclosure 4 (ANOVA; $P > 0.05$).

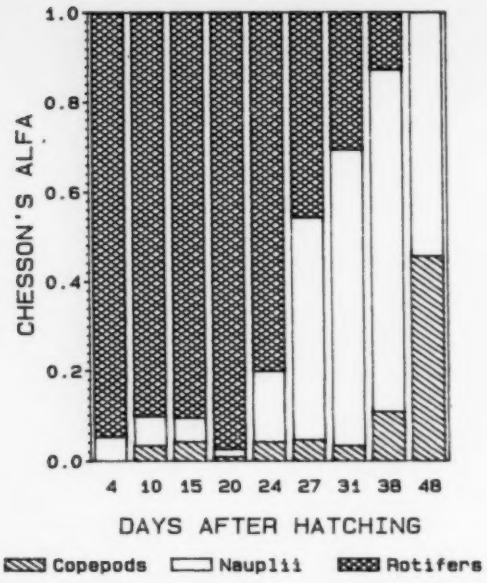

FIG. 8. Chesson's α for the most important larval prey (mean of all experimental enclosures).

Rotifers were most abundant in the bottom samples at all times, while nauplii were generally most abundant in the surface samples (Fig. 9b). There seemed to be no major vertical migration of zooplankton during the sampling period, although an aggregation of plankton in the bottom sample at 14:00 was evident from Fig. 9b.

The number of prey items per larval gut was lowest at 06:00, with mean numbers between 0.8 and 3.8/gut (Fig. 9c). These prey were also mostly digested. There were no differences in gut content with depth ($P > 0.05$).

Growth and Survival

On day 31, the larvae from the eight enclosures were not significantly different with regard to SL or dry weight (ANOVA; $P > 0.05$). After day 31, there were not enough larvae from all of the enclosures to compare statistically. Growth in SL and dry weight of pooled larvae is shown in Fig. 10, and Table 2 gives regression parameters of each enclosure.

The relationship between SL and dry weight of whole larvae from age 4 to 48 d was given by the regression equation

$$\log_{10}(W_{\mu g}) = 3.87 \times \log_{10}(SL_{mm}) - 0.897;$$

$$R^2_{corr.} = 0.88; N = 2419.$$

About 70% of the larvae had negative growth until day 10 (Fig. 11). This proportion was reduced to about 25% until day 20 and 5% until day 31. On day 48, almost 40% of the larvae had experienced a growth rate ≥ 6%/d.

The survival was quite similar in all enclosures until day 27 (ANCOVA; $P > 0.05$), with daily mortality rates (Z) between 0.00 and 0.05/d (mean = 0.02). However, from days 27–31 to days 41–45 the mortality increased considerably to between 0.23 and 0.40/d (Fig. 12). When the enclosures were emptied 4648 cod fry were collected, giving a total survival of about 0.6% over a period of 53–67 d.

Variation within and between Enclosures

Larval gut content was variable within enclosures (Fig. 13a), especially during the first 2 wk when feeding incidence was relatively low. Coefficient of variation (CV) decreased from 150% on day 4 to about 50% on day 22 and varied between 50 and 90% during the rest of the experiment. CV for dry weight within enclosures increased from about 13% on day 4 to about 60% on day 48, while CV's for population estimates were somewhat higher (Fig. 13a).

The CVs between enclosure means for plankton content, gut content, dry weight, and numbers of larvae are shown in Fig. 13b. CV for plankton increased from about 20% on day 4 to about 120% on day 48, while CVs for gut contents were relatively stable at about 20–50%. The CVs for larval population estimates were much higher than for larval weights, increasing from approximately 25 and 4%, respectively, on day 4 to about 130 and 40%, respectively, on day 48. The CV for larval abundance at end of the experiment was 71%. CV's of growth rates estimated from the SL and W regressions (Table 2) were 8.2 and 11.0%, respectively.

Discussion

The eight enclosures generally replicated well with respect to plankton content and larval feeding, growth, and survival. Larval growth was very low, in spite of relatively high plankton abundance. Mortality was low during the first month but increased dramatically during the last part of the experiment. Starvation was, in my opinion, the main reason for the low growth, which in this case resulted in the high mortality rates.

Hydrography

The salinity in the enclosures at release (>34) was high enough to avoid sinking of the larvae (Ellertsen et al. 1980; Yin and Blaxter 1987). Ammonia concentration was not measured, but the relatively high water exchange rate (about 100%/d) should eliminate any buildup of harmful substances. This was also suggested by the high oxygen levels. The low temperature in the experiment probably contributed to the low growth rate, compared with pond experiments. Although Campana and Hurley (1989) modelled a temperature optimum of 5.9°C for cod larvae from Nova Scotia, laboratory experiments indicated a higher temperature optimum (>10°C) for cod larvae from both the United States (Laurence 1978) and Norway (Opstad et al. 1989).

Feeding

Feeding incidence of cod larvae was high and comparable with reported values from field studies (Wiborg 1948; Ellertsen et al. 1981b, 1984; Kane 1984; Tilseth and Ellertsen 1984b) and mesocosm experiments (Ellertsen et al. 1981a; Gamble and Houde 1984; Kvenseth and Øiestad 1984). The stomach content was dominated by rotifers until day 24, despite abundant nauplii in the enclosures from day 10. Ellertsen et al. (1981a) also found that cod larvae preferred rotifers to nauplii, which they suggested were more difficult to catch. In contrast, in the enclosure experiment of Gamble and Houde (1984), cod larvae showed a clear preference for copepod nauplii, the dominating planktonic organism. Since the prey-catching capabilities of fish larvae are connected to larval size (Kane 1984), the slow larval growth in the present experiment may have delayed the change

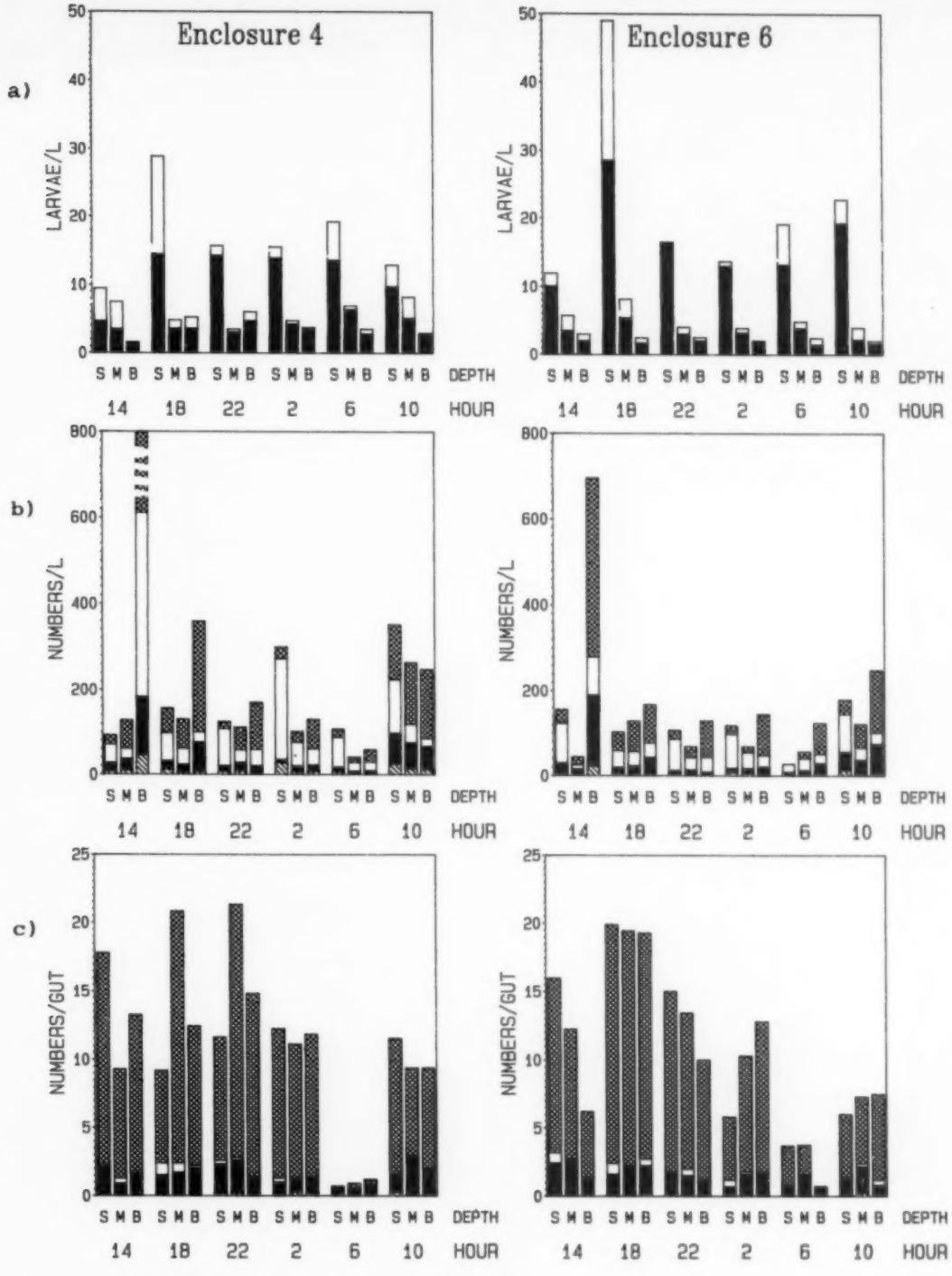

FIG. 9. Results from the day and night sampling, depths (S = surface samples, M = midwater samples, and B = bottom samples), and hour indicated. (a) Larvae/L: mean (black bars) + SD (open bars). (b) Mean numbers/L in the enclosures of rotifers (cross-hatched bars), copepod nauplii (open bars), rotifer eggs (black bars), and copepods (right-hatched bars). The bar representing the bottom sample of rotifers on 1400 has been truncated, and the actual value was 522/L. (c) Mean numbers/larval gut of rotifers (cross-hatched bars), copepod nauplii (open bars), rotifer eggs (black bars), and copepods (right-hatched bars).

TABLE 2. Linear weighted regression parameters (±SE) of SLs (N = 257–260/enclosure) and dry weights (N = 185–195/enclosure) by larval age.

	$\ln(SL_{mm})$ (age 4–31 d)			$\ln(W_{\mu g})$ (age 10–31 d)		
Enclosure	Intercept	Slope	$R^2_{corr.}$	Intercept	Slope	$R^2_{corr.}$
1	1.459 ± 0.01	0.0090 ± 0.001	0.60	3.353 ± 0.05	0.0437 ± 0.003	0.60
2	1.473 ± 0.01	0.0080 ± 0.001	0.53	3.509 ± 0.05	0.0360 ± 0.003	0.46
3	1.438 ± 0.01	0.0095 ± 0.000	0.64	3.352 ± 0.05	0.0421 ± 0.003	0.57
4	1.475 ± 0.01	0.0080 ± 0.001	0.62	3.466 ± 0.05	0.0369 ± 0.003	0.53
5	1.471 ± 0.01	0.0081 ± 0.001	0.54	3.471 ± 0.06	0.0373 ± 0.003	0.45
6	1.478 ± 0.01	0.0078 ± 0.001	0.54	3.388 ± 0.05	0.0426 ± 0.002	0.60
7	1.480 ± 0.01	0.0074 ± 0.001	0.46	3.579 ± 0.06	0.0309 ± 0.003	0.31
8	1.478 ± 0.01	0.0082 ± 0.001	0.55	3.449 ± 0.05	0.0401 ± 0.003	0.53
All	1.469 ± 0.00	0.0082 ± 0.000	0.53	3.447 ± 0.02	0.03782 ± 0.001	0.49

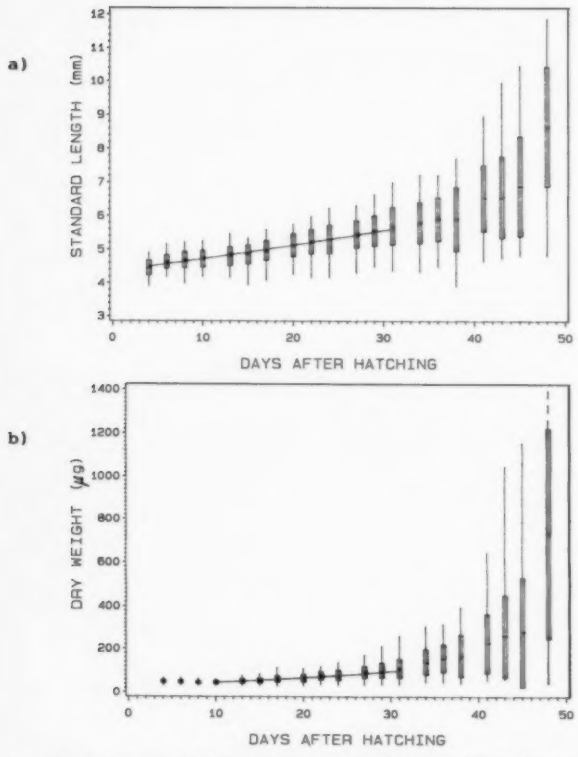

FIG. 10. (a) SLs and (b) dry weights during the experiment (mean, SD, range, and regression line indicated; mean of larvae from all experimental enclosures). The maximum weight on day 48 was 1887 µg.

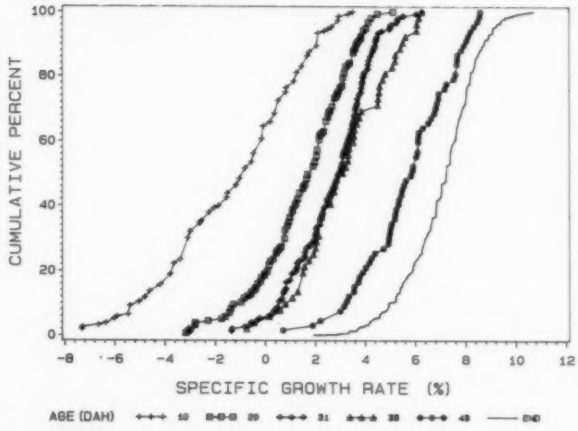

FIG. 11. Distribution (cumulative) of specific growth rates on some selected days during the experiment and at emptying of the enclosures (END).

from rotifers to larger and presumably more nourishing copepod nauplii.

Uneven distribution of zooplankton and larvae in the enclosures may have biased the electivity calculated from the net samples (O'Brien and Vinyard 1974). However, samples from the day and night station, with 5-L sampling volume, gave Chesson's $\alpha_{rotifers}$ about 0.90 for most of the samples, which compares well with the net-sample estimates of 0.97 and 0.80 from day 22 and day 24.

Although the feeding incidence was high, the mean values of 2–4 mostly digested rotifers per larval gut until day 10 indicate low food intake (Tilseth and Ellertsen 1984a). Gamble and Houde (1984) reported gut content to be about 6% of total dry weight of cod larvae, while only 2% or lower was observed after day 29 in the present experiment. The low food intake was, in spite of high zooplankton concentration in the enclosures (15–80 rotifers/L during the first 2 wk), higher than reported from several pond studies on cod larvae where growth rate was high (Øiestad 1985; Blom et al. 1991) and from the first-feeding areas of cod larvae in Lofoten, Norway (Ellertsen et al. 1987).

A partial segregation of larvae and rotifers was evident in the present experiment. Although Gamble (1985) suggested that food distribution patterns are important in larval feeding abilities, gut analysis from the day and night sampling gave no evidence of higher gut content in the deeper parts of the enclosures where the prey to larvae ratio was highest. There was no evidence for vertical migrations of larvae or prey from the day and night samples.

The abundance estimates of larvae obtained from samples collected with the Niskin bottle (day and night samples) should be examined with care due to the low sampling volume. However, the estimates of 6.4 and 7.2 larvae/L for enclosures 4 and 6, respectively, fit reasonably well with the estimates of 8.1 and 6.7 larvae/L obtained from the plankton net samples (average numbers from days 22 and 24). The higher weight of larvae

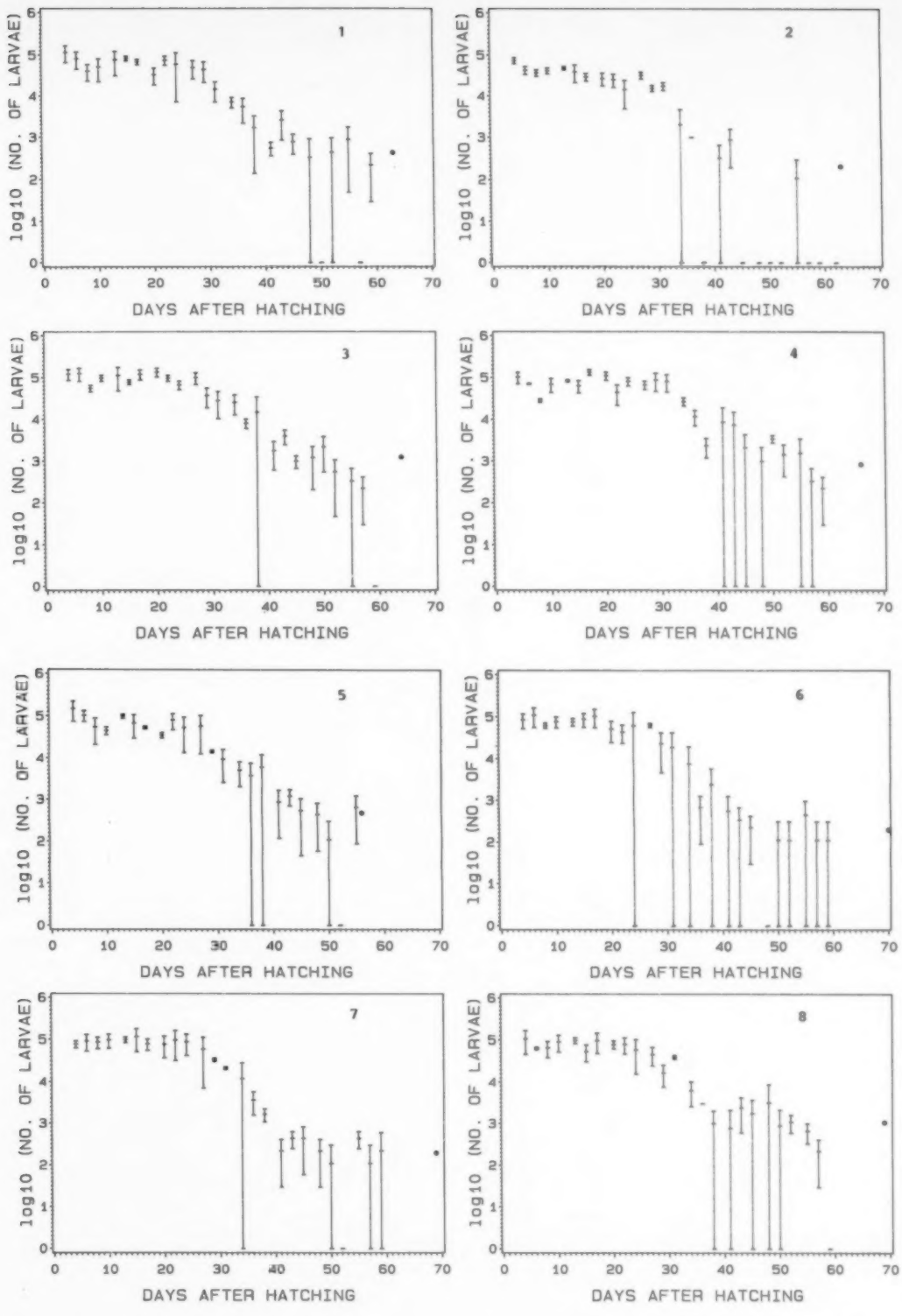

FIG. 12. Estimated survival in the eight experimental enclosures (error bars indicate SD). Solid circles denote actual numbers at emptying of the enclosures.

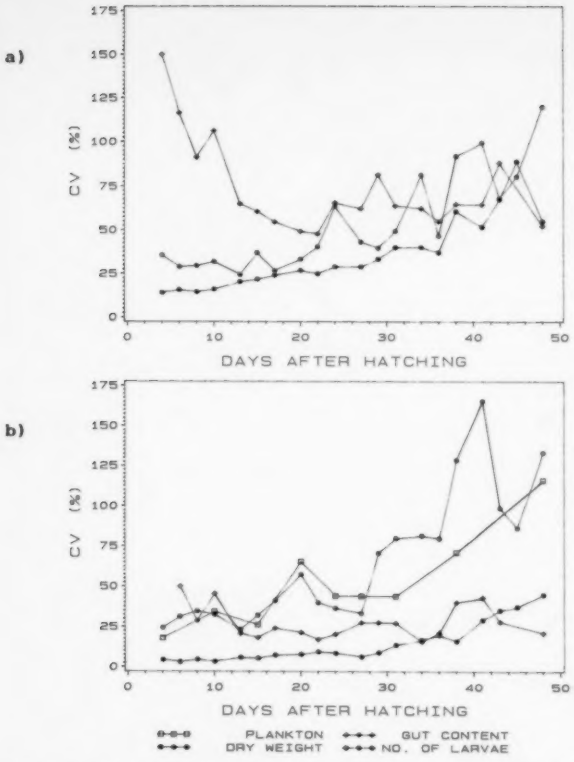

FIG. 13. CVs (a) within enclosures (mean of all enclosures) and (b) between enclosure averages. CVs were calculated for plankton and gut content (sum of numbers of rotifers, rotifer eggs, nauplii, and copepods), dry weight, and larval population estimates.

caught during night time in enclosure 6 suggests greater gear avoidance during daytime.

Intraspecific interactions caused by the relatively high larval stocking density could have reduced the feeding capabilities of the larvae. Larval densities ≥8/L strongly reduced growth and survival of sea bream (*Archosargus rhomboidalis*) at food concentrations of 1500 or 3000/L, while at food concentrations of 500/L, only survival was affected (Houde 1975).

Growth

Increments in SL during the period from release to day 31 were about 0.04 mm/d, much lower than reported by most other works on cod in both enclosures and laboratory and field studies (Gamble and Houde 1984; Øiestad et al. 1985b; Yin and Blaxter 1986; Bolz and Lough 1988; Campana and Hurley 1989). By contrast, Neilson et al. (1986) reported growth increments as low as 0.02 mm/d during the first 3 wk in their laboratory experiments with 5–10 zooplankton/mL.

About 80% of the larvae in these enclosures had a specific growth rate below 4%/d during the first month, compared with above 7%/d during the same period from pond studies (Øiestad 1985; Blom et al. 1991). Similarly, Gamble and Houde (1984) reported growth rates of cod larvae to be 10.0 and 10.4%/d in their two plastic bags (300 m^3), but in their small tanks (30 L), growth rates varied between 1.7 and 7.8%/d. The slow growth during the first weeks corresponded well with the amount of rotifers supplied to the enclosures during the first 3 wk (30–150 kJ/d), which would only support a growth rate of about 4%/d according to the growth model of Blom et al. (1991).

The specific growth rate distributions on day 31 and day 38 were similar, which implies a stagnation in growth at that time, or even a decrease in growth if a size-selective mortality was present.

The present experiment demonstrates that cod larvae are able to survive with growth rates of about 5%/d for as long as 2 mo, while Øiestad et al. (1985a) did not find any cod larvae with growth rates less than 9%/d on day 39. These values are much lower than the 12%/d Jones (1973) modelled as a minimum growth rate for cod and haddock larvae to survive in nature.

Survival

Mortality rates (Z) were quite low (0.00–0.05/d) until day 27. From that time, mortality increased significantly, with Z between 0.23 and 0.40/d. No clear reason for this increase in mortality could be found, and several factors could indeed be an influence. The increase in mortality coincided with a stagnation in growth and came after several days with decreasing gut content. This suggests that the larvae were starving, and it cannot be ruled out that also the nutritional quality of the rotifers and zooplankton was inadequate. Chemical analyses indicated that the type of enrichment food for rotifers used in the present experiment had 0.24% of highly unsaturated fatty acids (20:5 N-3 and 22:6 N-3) (Olsen et al. 1985), while the estimated requirement is around 1% (Watanabe 1982). Gamble and Houde (1984) observed a similar mortality pattern for cod larvae in 300-m^3 plastic bags around day 20, which they attributed to starvation. In laboratory experiments with cod larvae fed dry food, starvation was observed as late as day 35 (Garatun-Tjeldstø et al. 1986). Northern anchovy (*Engraulis mordax*) starved for 3 or 4 d had a survival until day 13 of 20 and 6%, respectively, as compared with 70% for fed larvae (Theilacker and Dorsey 1980). For juvenile cod (0.2 g wet weight), 1 wk without feeding reduced survival after 2 wk to 75% as compared with, 90% for fed fish, partly due to cannibalism (Folkvord 1991). Although cannibalism was not observed, it could have contributed to mortality in the late part of the experiment. In feeding experiments with juvenile cod, Otterå and Lie (1991) found that cannibalism accounted for about 28% of the mortality in their smallest size group (12.8 mg wet weight).

The high standard deviation of the abundance estimates of larvae was probably due to patchy distribution of larvae in the enclosures. Gear avoidance obviously increased with larval size and swimming speed, and thereby contributed to an underestimation of larval growth during the last part of the experiment and the underestimation of the larval population before emptying the enclosures. Growth or feeding estimates were not done for larvae after day 48 due to insufficient numbers. The largest larvae had also reached metamorphosis (about 12 mm SL and 1800 μg dry weight; Kvenseth and Øiestad 1984) at this time, and the sampling gear was therefore considered size selective.

Replication

The CVs for gut content among enclosures was surprisingly stable and low compared with the high and increasing variation in plankton content between enclosures. Population estimates between enclosures also had quite variable and increasing CVs, partly due to sampling. Estimates of larval weight between

enclosures were, however, similar, at least during the first month when CVs were below 10%.

Although some of the CVs were quite large, the enclosures had similar plankton content, and gut content, growth, and survival among the larvae. It was generally not possible to find any significant differences between the enclosures. This emphasizes the usefulness of such enclosure experiments in performing studies of larval ecology as stated by Gamble (1985) and de Lafontaine and Leggett (1987a). The high variation within enclosures also points out that replication is absolutely necessary if reliable conclusions are to be drawn.

Acknowledgements

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Year-Class Fluctuations of Vendace (*Coregonus albula*) in Lake Pyhäjärvi, Southwest Finland, during 1971–90

Harri Helminen

Department of Biology, University of Turku, SF-20500 Turku, Finland

Heikki Auvinen

Finnish Game and Fisheries Research Institute, Fisheries Research Station for Eastern Finland, SF-58175 Enonkoski, Finland

Arto Hirvonen and Jouko Sarvala

Department of Biology, University of Turku, SF-20500 Turku, Finland

and Jorma Toivonen

Finnish Game and Fisheries Research Institute, Fisheries Research Station for Eastern Finland, SF-58175 Enonkoski, Finland

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Many recent studies have stressed density-dependent regulation mechanisms in oscillating vendace (*Coregonus albula*) populations. Long-term studies on the population dynamics of vendace have been hampered by the difficulty of obtaining reliable estimates of year-class sizes. Data collected from Lake Pyhäjärvi (Southwest Finland) during 1971–91 allowed relatively accurate analysis of vendace abundance changes. In the winters of 1979–91, daily catch statistics were collected from professional fishermen. Species, age, and size composition were analysed from regular catch samples. We estimated the size of the vendace population from the decrease in the CPUE during 10 winters. For the remaining 10 years, estimates were based on the relationship between the numbers of a year-class in autumn and mean individual growth rate. A 2-yr cycle was the most conspicuous feature of the data implying density-dependent regulation. We suggest that in Lake Pyhäjärvi the basic model producing a persistent 2-yr cycle is the asymmetrical food competition between age groups.

De nombreuses études récentes ont mis l'accent sur des mécanismes de régulation de la population en fonction de la densité chez le corégone blanc (*Coregonus albula*). La difficulté d'obtenir des valeurs estimées fiables des tailles des classes d'âge faisait obstacle aux études à long terme sur la dynamique des populations de cette espèce. Des données collectées dans le lac Pyhäjärvi (sud-ouest de la Finlande) durant la période 1971–1991 ont permis une analyse relativement précise des changements touchant l'abondance du corégone blanc. Au cours des hivers 1979–1991, les données sur les prises quotidiennes ont été obtenues des pêcheurs professionnels. La composition du point de vue de l'espèce, de l'âge et de la taille a été analysée à partir d'échantillonnages réguliers des prises. Nous avons estimé la taille de la population de corégones blancs à partir d'une diminution des PUE durant 10 hivers. Pour les 10 autres années, les estimations ont été fondées sur la relation entre l'effectif d'une classe d'âge en automne et le taux de croissance individuel moyen. La caractéristique la plus évidente a été l'existence d'un cycle de deux ans laissant supposer l'existence d'une régulation liée à la densité. Nous croyons que dans le modèle de base du lac Pyhäjärvi, le cycle de deux ans persistant est produit par la compétition alimentaire asymétrique entre les groupes d'âge.

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Vendace (*Coregonus albula*) is the most important fish species in the commercial inland fisheries of Finland. Variation in year-class strength is common in vendace (Hamrin and Persson 1986; Viljanen 1986; Auvinen 1988). Often there are distinct oscillations with varying periodicity in different populations: in some lakes the time period does not exceed 2 yr whereas in others it can be as much as 5 yr (Viljanen 1986). Several hypotheses concerning the possible reasons for these fluctuations have been proposed (see Viljanen 1986), but no agreement has yet been reached. However, several recent studies have stressed density-dependent regulating mechanisms in oscillating vendace populations (Hamrin and Persson 1986; Viljanen 1986, 1988; Sandlund et al. 1991).

The difficulties in detecting density dependence in a sequence of population estimates are well known (e.g., Pollard and Lakhani 1987; Turchin 1990; Fogarty et al. 1991). Yet, because experimental tests of density dependence are impracticable on a large scale, further advances in understanding population regulation of vendace must come from detailed observations spanning long temporal scales. In this paper, we analyse population dynamics of vendace using data collected from Lake Pyhäjärvi (Southwest Finland) during 1971–90. We describe year-class

TABLE 1. Catch and growth data from Lake Pyhäjärvi for 1971–90. Size = annual year-class size estimates millions of 0+ vendace in the beginning of the winter fishing period; Catch = actual catch of millions of 0+ vendace in the subsequent winter; Effo = fishing effort as numbers of seine hauls during winter; Wei = mean weight of 0+ vendace in the beginning of the fishing period based on samples taken by the Finnish Game and Fisheries Research Institute (FGFRI) and the University of Oulu (sample size and standard deviation are given in parentheses after the sample mean); Per = length of linear growth period in days.

		Catch data		Growth data			
Year-class	Size	Catch	Effo	FGFRI	Univ. of Oulu	Wei	Per
1971	14.8	—	—	—	—	—[a]	166
1972	11.8	—	—	—	—	—[a]	157
1973	22.6	—	—	12.8 (70; —)	12.0 (73; 2.4)	12.4	165
1974	4.8	—	—	25.8 (176; —)	23.8 (29; 2.7)	24.8	164
1975	26.5	—	—	10.6 (87; —)	10.7 (84; 1.6)	10.7	182
1976	11.4	—	—	17.0 (90; —)	16.7 (41; 2.7)	16.9	149
1977	14.8	—	—	18.0 (141; —)	16.8 (98; 2.3)	17.4	172
1978	11.8	—	—	—	17.5 (98; 2.3)	17.5	156
1979	33.5	32.2	819	5.1 (135; —)	5.2 (98; 0.2)	5.2	163
1980	7.3	6.7	982	20.2 (105; —)	—	21.3	163
				22.3 (77; 3.6)	—		
1981	15.3	5.0	513	16.9 (94; 2.0)	16.4 (40; 2.0)	16.7	168
1982	3.6	2.2	431	22.5 (176; 2.7)	24.1 (36; 3.5)	23.3	159
1983	21.6	17.1	866	13.4 (142; 1.6)	12.9 (97; 1.3)	13.2	171
				13.3 (99; 1.7)	—		
1984	4.8	4.6	872	24.7 (101; 2.9)	22.3 (77; 2.3)	23.5	182
1985	19.9	18.0	964	14.8 (273; 2.0)	14.0 (99; 1.8)	14.4	154
1986	13.5	11.8	954	17.7 195; 2.4)	18.6 (95; 2.3)	18.2	175
1987	11.8	11.1	1046	18.5 (189; 2.5)	17.5 (48; 2.2)	18.0	162
1988	15.6	14.4	853	15.5 (129; 1.7)	15.2 (49; 1.1)	15.6	163
				16.0 (50; 1.4)[b]	—		
				15.8 (50; 1.4)[b]	—		
1989	13.6	12.7	734	20.1 (50; 4.1)[b]	—	19.6	189
				19.0 (31; 4.0)[b]	—		
1990	4.4	4.0	293	26.2 (50; 3.9)[b]	—	26.7	188
				27.1 (40; 4.1)[b]	—		

[a]Growth rates calculated on the basis of gill net samples in August.
[b]Samples taken by the authors.

fluctuations in this lake and theoretically examine the underlying causes for these fluctuations.

Material and Methods

Study Area

Lake Pyhäjärvi is in southwestern Finland (60°54′–61°06′N, 22°09′–22°25′E), about 70 km north of Turku. With its few islands and relatively straight shoreline, Lake Pyhäjärvi is unusually open for a Finnish lake. It is quite large (area 154 km^2), but shallow (mean depth 5.4 m; maximum depth 25 m): 94% of lake area is less than 7 m deep. The quality of water, nutrient concentrations (total P between 12 and 19 $mg \cdot m^{-3}$), and primary production (between 26 and 56 g $C \cdot m^{-2} \cdot yr^{-1}$) of the lake are described in more detail in Sarvala and Jumppanen (1988).

Vendace were introduced into Lake Pyhäjärvi during 1948–52, and substantial catches occurred for the first time in 1957. The present population maintains itself wholly by natural reproduction. Vendace in Lake Pyhäjärvi grow rapidly; young fish usually attain a length of 13–16 cm during the first year and 19–22 cm during the second year. Females reach maturity in the fall of their second year (Sarvala et al. 1988; Helminen et al. 1990; Helminen et al. 1992; Sarvala et al. 1992a).

This vendace stock is now exploited by more than 30 commercial fishermen, who fish with gill nets (4.8-m-high nets of 12- to 20-mm mesh from knot to knot; 50–80 nets per man) during the summer, with fyke nets (maximum catching height 4 m, mesh size of the cod end 10 mm, mesh size of the leader 15–30 mm) in the late autumn, and with seines during the winter (Sarvala et al. 1992b). The type and size of the winter seines used for vendace are described in Sarvala et al. (1992b).

The major proportion of the total vendace catch is taken with seine nets in winter when the lake is ice-covered. The winter seining season usually starts in the beginning of December and lasts up to the second half of March or April. In the 1980s, there have been five to nine seine crews, each consisting of four to six men. Due to the homogeneous shape of the lake the catch effort is distributed apparently at random over most of the lake (Helminen et al. 1993). Vendace catches have been as large as 2000 $kg \cdot d^{-1}$ per seine crew. Neither seines nor trawls are used during the open-water season. Over 90% of the 0+ vendace are usually removed in their first winter (Helminen et al. 1992).

Catch Data

We obtained daily seine catch records for the winters of 1979–91 from each seine crew and the most important fish agent (for more details, see Helminen et al. 1992). The total fishing period was divided into 20 intervals of equal fishing effort and the catches for these intervals were calculated on the basis of daily catch statistics (Table 1).

The Finnish Game and Fisheries Research Institute (Fisheries Division) has taken one or two composite samples from winter seine net catches in each winter fishing period from 1970 to the

TABLE 2. Catch composition (y) in years when estimates of vendace year-class size were based on survey depletion data: $y = 1/(1 + a \cdot \mathrm{EXP}(b \cdot x))$. x = cumulative effort, y = proportion of 0+ vendace in the catch.

Year-class	Value of a	Value of b	Source
1979	0.04504	0.00369	Seine gang plus two samples
1980	0.24466	0.00135	Regression[a]
1983	0.04368	0.00157	Helminen et al. 1993
1984	0.21735	0.00318	Regression[a]
1985	0.00370	0.00380	Regression[a]
1986	0.03123	0.00274	Regression[a]
1987	0.04979	0.00297	Regression[a]
1988	0.03610	0.00217	Own data
1989	0.04097	0.00378	Helminen et al. 1993
1990	0.03606	0.01350	Own data

[a]Estimated from equations (2) and (3); see text.

present and analysed the age and size compositions of these catches by species for the winters of 1970–89. Furthermore, in winters 1979–1980 and 1983–1984 two seine crews separated and enumerated older age groups out of their total daily vendace catches. Catch composition data for days when the total catch was more than 800 kg were omitted because the sorting was likely to be incomplete. Supplementary data (one composite sample per winter) for the winters of 1973–87 were also available (E.A. Lind, University of Oulu, Oulu, Finland, unpublished data).

A two-stage sampling strategy was applied from December 1988 onwards (Helminen et al. 1992). The primary units were sleigh loads of unequal size (one or two loads per seine haul) and the secondary units were clusters (a fish box containing 12 kg of fish) of equal size. The number of the primary units varied from two to four and the number of secondary units from one to two. All fishes in each box were sorted by species, age, or size groups, weighed, and subsampled for individual measurements. Ages were determined from the scales. To ensure that the age designation of a cohort did not change during the winter fishing season, we counted ages from the hatching of larvae at the ice-out in April or May. From December 1988 to April 1989, we sampled twice a month, but from December 1989 to March 1990, we sampled monthly. In the winters of 1990–91, we sampled again twice a month.

In years for which the catch composition data were available through the whole winter (year-classes 1979, 1983, and 1988–90) the effect of varying catch compositions on the final estimates was examined by fitting a curve to the observed samples. This curve could be derived from the difference in capture probabilities of different age groups:

$$(1) \qquad y = 1/(1 + a \cdot \mathrm{EXP}(b \cdot x))$$

where x = cumulative effort during the fishing period, y = proportion of 0+ vendace in the catch, a = the ratio of proportions (others/vendace 0+) in the catch at the beginning of the fishing period, and $b = \log_e p(0+) - \log_e p(\text{others})$ where p is the probability of capture in one seine haul. The fitting was done with the least squares method (Helminen et al. 1993). Parameter values thus obtained are shown in Table 2.

For the remaining years, the seasonal change in catch composition was calculated from the same equation using values for the parameters a and b derived from equations (2) and (3):

$$(2) \qquad b = 1.4424 \cdot (1 - q) \qquad (R^2 = 0.953;\ P = 0.0008;\ n = 5)$$

where q = regression coefficient from the relationship $\log_{10}$ (total catch) = constant + q·(cumulative effort). The

TABLE 3. Data and methods available for different year-classes.

	Catch data		Population estimation methods	
Year-class	Growth samples (n)	Composition samples (n)	DeLury[a]	Regression based on the growth rate
1971	1	–	–	+
1972	1	–	–	+
1973	2	–	–	+
1974	2	–	–	+
1975	2	–	–	+
1976	2	–	–	+
1977	2	–	–	+
1978	1	–	–	+
1979	2	63	+	–
1980	2	2	+	–
1981	2	2	–	+
1982	2	2	–	+
1983	3	90[a]	+	–
1984	2	2	+	–
1985	2	2	+	–
1986	2	2	+	–
1987	9	2	+	–
1988	6	17[b]	+	–
1989	6	25[b]	+	–
1990	8	21	+	–

[a]Helminen et al. (1993).
[b]Helminen et al. (1992).

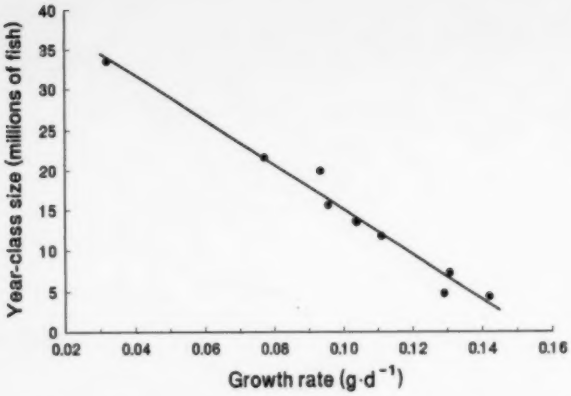

FIG. 1. Relationship between the numbers and the mean individual growth rate (mass at the end of the period (g)/length of the period (d)) of a year-class in the autumns of 1979, 1980, and 1983–90.

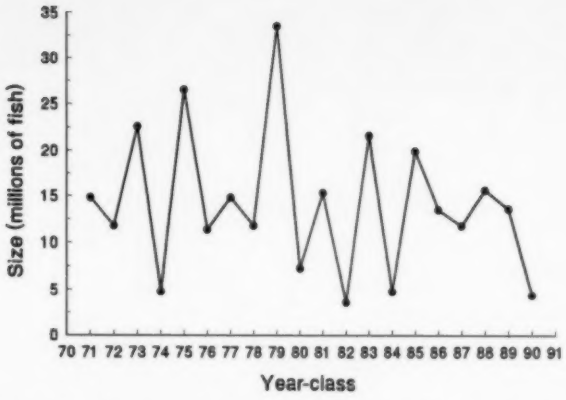

FIG. 2. Year-class strength of vendace (numbers of 0+ fish) in the autumns of 1971–90 in Lake Pyhäjärvi.

term $(1-q)$ thus denotes the proportion of the stock that is fished up in every haul. The parameter a was obtained as the maximum likelihood estimate of a in the regression equation

$$(3) \qquad \log_e(1/\text{prop} - 1) = a + b\cdot(\text{cumulative effort})$$

where prop = the proportion of age 0+ vendace in the total coregonid catch.

Information concerning data and methods that were available for estimating the sizes of different year-classes of vendace during 1971–90 is summarized in Table 3. Growth data were derived from the mentioned catch composition samples (Table 1). Mean weight of 0+ vendace at the beginning of the winter fishing period was calculated as an average for all samples available. The standard deviation of weight was found to be linearly related to the mean weight:

$$(4) \qquad \text{SD} = 0.131\cdot\text{mean} \qquad (R^2 = 0.960;\ P << 0.001;\ n = 28).$$

Population Estimates

In the 1980s, the winter seine net fishing in Lake Pyhäjärvi was extremely efficient: e.g., for the 1988 vendace cohort the total instantaneous mortality rate was 4.5 from autumn 1988 to autumn 1989 (Helminen et al. 1992). Therefore, except in 1981 and 1982, we could estimate the year-class size in the autumns of 1979–90 from the decrease in the catch-per-unit-effort (CPUE) during the winter with methods described in detail in Helminen et al. (1993).

Removal methods were not applicable for the winters of 1981–82 and 1982–83 because the winter fishery did not significantly reduce the CPUE during the fishing season. The year-class sizes for these years and for 1971–78 were estimated from the relationship between the numbers of a year-class in autumn and mean individual growth rate. The mean individual growth rate (mass at the end of period (grams)/length of the period (days)) was calculated for the linear growth period, which was considered to start when water temperature reached 10°C and to stop when water temperature decreased below 5°C (Table 1; see Helminen et al. 1990). The relationship between age 0+ vendace growth rate (GR) and 0+ abundance (A) was linear (Fig. 1):

$$(5) \qquad A = 4.28 \times 10^7 - 2.770 \times 10^8\cdot\text{GR} \qquad (R^2 = 0.976;\ P << 0.001;\ n = 10).$$

Statistical Analysis

Visual examination of vendace abundance fluctuations suggested that year-class sizes were alternating between high and low values (Fig. 2). To test whether this two-point oscillation was statistically significant, we calculated the autocorrelation function (ACF) for these data (Box and Jenkins 1976). To extend the idea of autocorrelation, which measures the correlation of terms of the series separated by assigned numbers of terms, we also calculated the partial autocorrelation function (PACF) where dependence on the intermediate terms has been removed (Box and Jenkins 1976). The 95% confidence levels for both ACF and PACF were calculated from Bartlett's formula (Box and Jenkins 1976). These levels could be used for judging whether the autocorrelations and partial autocorrelations are effectively zero after some specific lag q.

Results

The vendace population of Lake Pyhäjärvi exhibited variations in year-class strength generally characterized by a 2-yr cycle of alternating strong and weak year-classes (Fig. 2). The long-term arithmetic mean was 14.2×10^6 vendace age 0+ in the beginning of the winter fishing period, but the difference between the highest and lowest values was nearly 10-fold: the maximum was 33.5×10^6 (in 1979) and the minimum was 3.6×10^6 (in 1982). The CV for these data was 54.8%, reflecting the variable character of this time series. There was no significant trend over the study period.

Year-class strengths of successive years were negatively correlated (Fig. 3). This was also evident in the shape of the estimated ACF which was characterized by correlations that alternate in sign, a negative spike at lag 1 and its odd multiples (1, 3, 5, . . .) and a positive spike at lag 2 and its multiples (2, 4, 6, . . .), and that tend to damp out with increasing lag (Fig. 4a). However, only the correlation at lag 1 was significantly different from zero: ACF(1) = −0.572 and the lower 95% confidence limit was −0.406. The results from the PACF confirmed this pattern: the coefficient of partial correlation with lag 2 was only 0.064 (Fig. 4b). The estimated ACF and PACF

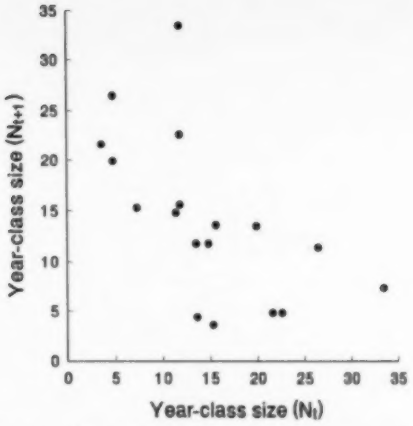

FIG. 3. Relationship of the year-class size in year t (N_t; millions of fish) to that in year $t + 1$ (N_{t+1}) in a series of autumn stock estimates of 0+ vendace in Lake Pyhäjärvi.

thus indicate a highly significant periodic component in vendace year-class fluctuations with lag 1.

Discussion

In population monitoring studies over several generations, identifying the density-dependent signal from the environmental noise is of fundamental importance to ecology (Hassell et al. 1989). Estimation of time trends in population abundance is thus an essential component in fisheries management when addressing regimes on a truly scientific basis (e.g., Peterman and Bradford 1987).

Long-term studies on the population dynamics of vendace have been hampered by the difficulty of obtaining reliable estimates of year-class sizes. We had a rare opportunity to investigate a lake where the commercial winter fishing is so efficient and so well documented that we could estimate the abundance of the vendace stock directly from the declining daily catches during the main fishing season. The daily seine catch records from the winters of 1979–91 obtained from the five to nine seine crews covered practically all vendace catches during winter fishing seasons; four crews had fished throughout the whole period which improves the comparability of the data. In addition, the figures from the records of the most important fish agent at Lake Pyhäjärvi were consistent with those reported by the individual fishermen.

The fluctuation patterns we found have similarities with other long-term investigations of planktivorous fish in freshwater ecosystems. For example, Townsend et al. (1990) analysed published 2-yr cycles in recruitment of roach (*Rutilus rutilus*) in two small eutrophic lakes in which the explanations offered by the investigators were different. In one lake (Alderfen Broad; Cryer et al. 1986) where food supply was the limiting factor, density-dependent loss of fecundity could produce the observed stable limit cycles, while in the other lake (Slapton Ley; Wyatt 1988), a year of good recruitment was followed 2 yr later by another, when the previous prolific year-class attained sexual maturity. Simple population models (Townsend et al. 1990) showed that both mechanisms could indeed produce cycles when the annual survival rate was lower than about 50%. Sur-

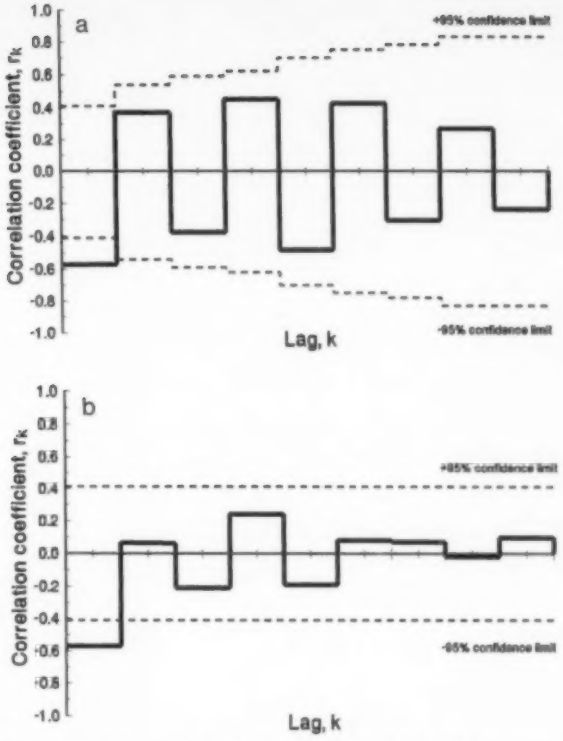

FIG. 4. (a) Autocorrelation function (ACF) and (b) partial autocorrelation function (PACF) with 95% confidence limits for the 20-yr record of year-class fluctuations of vendace in Lake Pyhäjärvi.

vival rates of roach in these lakes were unusually low, probably as a result of eutrophication (Townsend and Perrow 1989).

In Lake Pyhäjärvi, due to efficient winter fishing, the mean annual survival rates of vendace seemed to be much lower than 50% (Helminen et al. 1992, 1993). Therefore, cycling population dynamics are very probable for vendace in Lake Pyhäjärvi. Theoretically, a particularly strong and persistent cycle is to be expected when inter-age-group competition for food is clear because the delayed feedback has the potential to produce a 2-yr cycle, which will be reinforced by the period of 2 yr from hatching to maturity (Townsend et al. 1990).

In Lake Pyhäjärvi, strong and weak year-classes of vendace alternate regularly. Provided that the total mortality rate during the second year of vendace life does not vary appreciably between years, a strong year-class should result in a strong spawning stock. If the spawning population each year were made up entirely of newly mature females, then there would be two almost isolated stocks within the lake, one stock spawning in even-numbered years and another one spawning in odd-numbered years. In this situation, there would be a direct relationship between the number of spawning fish and the catch of their progeny. A small spawning population would lead to a small catch; a large spawning population would lead to a large catch.

However, in Lake Pyhäjärvi the reproductive isolation of the stocks of alternate years was far from complete. Although the spawning stock of vendace in Lake Pyhäjärvi mainly consists of age 1+ fishes, other age groups may in certain years also have a significant role in reproduction. The contribution of the

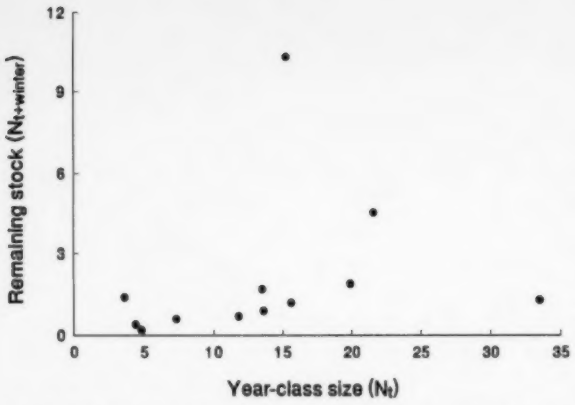

FIG. 5. Relationship of the year-class size in the autumn before the first winter fishing period (N_t; millions of fish) to the year-class size remaining after the winter fishery ($N_{t+\text{winter}}$) for the year-classes 1979–90.

age 1+ females to the total estimated number of eggs laid in the whole population was 56% in 1983, 85% in 1987, 80% in 1988, and 54% in 1989 (Sarvala et al. 1992a). In 1989, 1.1% of the females matured in their first autumn. Although their absolute fecundity was low, they produced 32% of the total number of eggs spawned (Sarvala et al. 1992a); these, however, were much smaller than normal and their quality may have been inferior.

Viljanen (1988) found in eastern Finnish lakes that the density of newly hatched vendace larvae reflects the spawning stock size but does not indicate the strength of the new year-class. By contrast, the density of 3-wk-old larvae was found to have a strong positive correlation with the future strength of that year-class. Auvinen (1988) concluded that the factor determining the year-class success between hatching and the age of 3 wk could be the availability of food for the larvae. However, in Lake Pyhäjärvi the food conditions for coregonid larvae are so good that mortality through starvation during the larval period is unlikely (Sarvala et al. 1988), and therefore, a strong population of newly hatched larvae might result in a large year-class size the following autumn.

On the other hand, due to the very intensive winter fishing, the number of fish in a vendace year-class surviving the first winter seems not to be predictable from the year-class size before the fishing period (Fig. 5). Therefore, although we do not have any independent estimates of the actual spawning stock, it seems very unlikely that a strong year-class would always result in a strong spawning stock. Thus the ultimate cause for the regular 2-yr population cycle of vendace cannot be the size of the spawning stock.

Our data showed that in years of good recruitment the young fish grew poorly and their growth rate could be directly predicted from their year-class strength (Fig. 1). The growth of the adult vendace in its second year depends on its starting weight at age 1 and the year-class strength of young vendace in that year, but not on the strength of its own year-class (J. Sarvala et al., unpublished data). This is most probably due to a depression of prey abundance by the abundant juvenile stock later in summer. Helminen et al. (1990) found that the vendace population of Lake Pyhäjärvi has the potential to influence the population dynamics of its main prey species, especially the cladoceran *Bosmina coregoni*. Within the vendace population, the 0+ vendace were the major consumers of zooplankton during most of the summer (Helminen et al. 1990). Although the growth of the young-of-the-year vendace is strongly density dependent, the food consumption of the whole vendace population in the lake is not constant, but reflects variations in recruitment (Helminen and Sarvala 1993). Helminen and Sarvala (1993) thus suggested that variations in total zooplanktivory before spawning (August–October) could be the decisive factor in maintaining population fluctuations of vendace in Lake Pyhäjärvi.

The ultimate cause producing a persistent 2-yr cycle of vendace in Lake Pyhäjärvi would then be the asymmetrical food competition between age groups (Hamrin and Persson 1986; Townsend et al. 1990). This hypothesis is based on one central argument, namely that small vendace will have a competitive advantage during summer due to their lower metabolic requirements and the low abundance and mean size of available resources (Hamrin and Persson 1986). A strong new year-class competes heavily with the 1-yr-old vendace, resulting in a low reproductive output of the latter. The negative feedback in the population dynamics of vendace in Lake Pyhäjärvi is clear (Fig. 3 and 4), and the growth relationships of both the young and adult vendace are consistent with the food competition hypothesis, but more detailed monitoring surveys with very high quality field data are needed before the exact mechanism behind this model can be solved.

The significance of other biotic and abiotic factors (such as predation and water temperature during the larval phase) in adjusting the final year-class strength of vendace should also be clarified. It seems that their interaction may sometimes perturb the 2-yr cycle, as has apparently happened in recent years when the water temperature after hatching has been lower than normal and predation pressure harsher (H. Helminen and J. Sarvala, unpublished data). Their contribution to the observed periodic year-class fluctuations of vendace in Lake Pyhäjärvi could, however, only be adjusting, not decisive, since they do not oscillate regularly but are either stochastic or correlated with stochastic events (e.g., Böhling et al. 1992).

Acknowledgements

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Inhibition of Chorion Expansion and Preservation of Fertility in Goldfish (*Carassius auratus*) Eggs by Protease Inhibitors

Sheau-Yu Hsu and Frederick William Goetz

Department of Biological Sciences, University of Notre Dame, Notre Dame, Indiana IN 46556, USA

Hsu, S.-Y., and F.W. Goetz. 1993. Inhibition of chorion expansion and preservation of fertility in goldfish (*Carassius auratus*) eggs by protease inhibitors. Can. J. Fish. Aquat. Sci. 50: 932–935.

The in vitro effects of protease inhibitors on chorion expansion and the fertility of goldfish (*Carassius auratus*) eggs were investigated. Expansion of the chorion in ovulated eggs was blocked by the serine protease inhibitors benzamidine and soybean trypsin inhibitor when they were held in a goldfish Ringer solution. Eggs in which chorion expansion was inhibited retained their fertility for 30 min when held in this solution. In contrast, ovulated eggs lost their fertility in freshwater even in the presence of a protease inhibitor. The results suggest that chorion expansion and loss of fertility in water-activated goldfish eggs may involve proteolysis which was blocked by benzamidine and soybean trypsin inhibitor. Moreover, the blockage of chorion expansion may be pivotal to the preservation of fertility in eggs held in Ringer's solution with soybean trypsin inhibitor. The development of this solution provides a means to temporarily hold ovulated goldfish eggs in a viable state outside the female and will permit further studies on fertilization in this species.

Les effets in vitro des inhibiteurs des protéases sur le développement du chorion et la fécondité des oeufs de poisson rouge (*Carassius auratus*) ont été étudiés. Le développement du chorion dans les oeufs ovulés était bloqué par les inhibiteurs des protéases à sérine, benzamidine et inhibiteur de la trypsine du soja, lorsque les oeufs étaient gardés dans une solution de Ringer pour poisson rouge. Les oeufs dans lesquels le développement du chorion était inhibé gardaient leur fécondité pendant 30 min dans cette solution de Ringer. À l'opposé, les oeufs ovulés ont perdu le fécondité dans l'eau douce même en présence d'un inhibiteur des protéases. Les résultats donnent à entendre que le développement du chorion et la perte de fécondité dans les oeufs de poisson rouge activés par l'eau peuvent faire intervenir la protéolyse qui était bloquée par la benzamidine et l'inhibiteur de la trypsine du soja. De plus, le blocage du développement du chorion peut être capital pour la conservation de la fécondité des oeufs gardés dans la solution de Ringer avec l'inhibiteur de la trypsine du soja. La mise au point de cette solution met à notre disposition un moyen de conserver temporairement les oeufs ovulés de poisson rouge dans un était viable à l'extérieur de la femelle et permettra de pousser plus loin d'autres études sur le fécondation chez cette espèce.

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Past research on the in vitro preservation of fish eggs prior to fertilization has focussed on the composition and temperature of the holding media (Stoss 1983; Erdahl et al. 1987). In salmonids, eggs remain fertilizable in the female for weeks after ovulation. The eggs of salmonid fish can also be held in various types of media after being stripped but remain fertilizable for a much shorter period of time (Billard and Jalabert 1974; Stoss 1983; Stoss and Donaldson 1983). However, in most other fish, especially warmwater species, the ovulated eggs become overmature and lose fertility after only a short period inside the female. Eggs removed from these species start to undergo the cortical reaction when held in isotonic Ringer's solution or freshwater (Yamamoto 1961). Few studies have been conducted to investigate this loss of fertility, and generally, eggs must be fertilized as soon as possible to insure a high fertilization rate once they are ovulated.

The expansion of the chorion in fish eggs is presumably due to the activation of the cortical reaction, a process in which the cortical granules are exocytosed into the perivitelline space following fertilization. An osmotic influx is then initiated and the chorion layer elevated away from the egg plasma membrane. The cortical reaction can also be induced by various physical (Yamamoto 1961) and chemical agents (Iwamatsu 1989). In the Japanese medaka (*Oryzias latipes*), it has been shown that this reaction may involve an increase in cytoplasmic calcium, pH, and possibly phosphatidylinositol turnover in the eggs (Gilkey 1983; Iwamatsu 1989).

In a preliminary study, we found that the chorion expansion of goldfish (*Carassius auratus*) eggs ovulated in vitro was blocked by several protease inhibitors. A subsequent study was initiated to characterize the effect of these protease inhibitors on chorion expansion in eggs ovulated in vivo. The development of this solution not only provides a means to temporarily hold ovulated goldfish eggs in a viable state outside the female but will also facilitate studies on fertilization and egg activation in fish.

Methods and Materials

Experimental Animals and in vitro Incubations

Gravid goldfish (30–50 g) were purchased from Ozark Fisheries (Stoutland, MO) prior to the reproductive season and maintained under long photoperiods (16 h light : 8 h dark) in circular flow-through tanks supplied with 12.5°C well water.

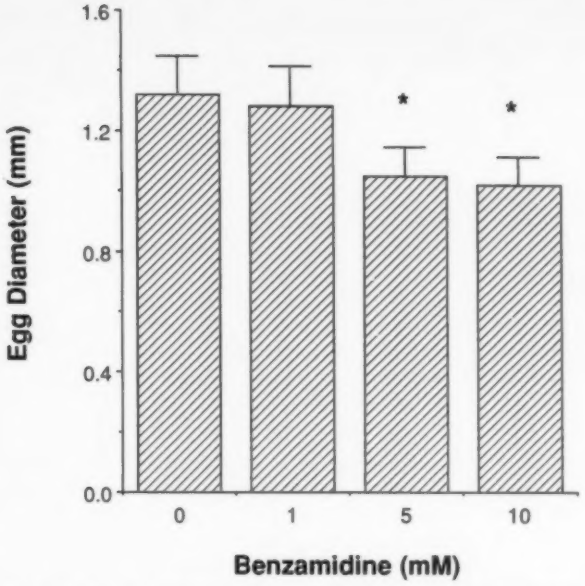

FIG. 1. Effects of benzamidine concentration on chorion expansion 2 h after stripping ($N = 6$). *Significantly ($P < 0.05$) different from control.

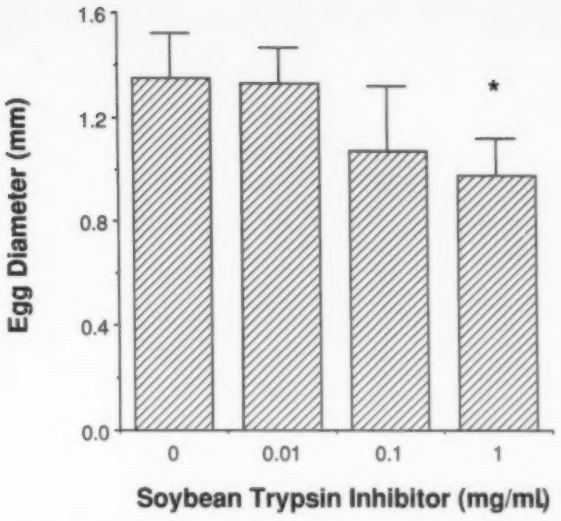

FIG. 2. Effects of soybean trypsin inhibitor concentration on chorion expansion 2 h after stripping ($N = 6$). *Significantly ($P < 0.05$) different from control.

Experiments were conducted during the reproductive season from mid-April to late June. Sexually mature females were chosen based on the size of the oocyte, sampled in vivo as described by Ranjan and Goetz (1987). Fish with oocytes greater than 1 mm in diameter were induced to undergo ovulation by a combination of warm temperature (20°C) and the injection of human chorionic gonadotrophin (HCG, 5 IU/g body weight), as previously described (Kagawa et al. 1984). Generally, ovulation occurs in vivo 10–15 h after HCG injection. The ovulated eggs were stripped by gently pressing the abdomen of the fish. In the experiments on chorion expansion, the eggs were collected directly into an 82-mm petri dish containing 20 mL of modified goldfish Ringer solution (buffered with Tris, pH 7.3) (Kagawa et al. 1984) with/without test agents and incubated at room temperature. At the time of assay, egg diameter was measured under a dissecting microscope fitted with an ocular caliper. In the experiments on fertilization rate, the eggs in control groups were first collected in a petri dish and fertilized by the dry method, while the fish in all other treatments were fertilized in goldfish Ringer 30 min after collection. The fertilized eggs were then washed several times with goldfish Ringer and incubated in freshwater at room temperature. The fertilization rate was assayed 2 h after fertilization by observing cell cleavage under a dissecting microscope. The test agents, benzamidine and soybean trypsin inhibitor, were purchased from Sigma Chemical Co. (St. Louis, MO). Within the experiments conducted on each fish, duplicates of 50–100 eggs each were tested per treatment.

Data Analysis

Statistical significance was assessed using Student's *t*-test or one-way analysis of variance, followed by Tukey's multiple comparison test. Values of P of less than 0.05 were considered significant.

Results

Effects of Protease Inhibitors on Chorion Expansion in Ovulated Eggs

Due to the expansion of the chorion, the diameter of unfertilized eggs increased from approximately 1 mm to more than 1.25 mm in goldfish Ringer after 2 h of incubation. The increase in egg size was dosage-dependently inhibited by benzamidine (Fig. 1) and soybean trypsin inhibitor (Fig. 2). Egg diameters in treatments with greater than 5 mM benzamidine or 1 mg soybean trypsin inhibitor/mL were significantly lower than those of controls. Separation of the chorion layer from the egg membrane was not observed in eggs of these treatments.

Effects of Soybean Trypsin Inhibitor on Fertility of Ovulated Eggs

The effect of protease inhibitor on the fertility of eggs was further characterized with soybean trypsin inhibitor. The fertilization rate of control eggs was 98.3 ± 2.06% 2 h after fertilization (Table 1). In contrast, the eggs held in freshwater, freshwater and soybean trypsin inhibitor, or goldfish Ringer could not be fertilized after 30 min of incubation. However, when the goldfish Ringer was supplied with 0.5 mg soybean trypsin inhibitor/mL, the fertility of eggs was maintained after 30 min of incubation and the fertilization rate was not significantly different from that of controls. The size of fertilized eggs in the treatment containing goldfish Ringer and soybean trypsin inhibitor was 1.16 ± 0.1 mm at 2 h postfertilization, not significantly different from that of controls (1.12 ± 0.15 mm). In contrast, the eggs in other treatments expanded to more than 1.4 mm in diameter. To examine whether the eggs developed normally in the goldfish Ringer/soybean trypsin inhibitor treatment, development of embryos was examined 48 h after fertilization. At that time, 75.3 ± 14.2% of the eggs in the goldfish Ringer containing soybean trypsin inhibitor developed

TABLE 1. Inhibition of chorion expansion and preservation of fertility in goldfish eggs by soybean trypsin inhibitor (TI, 0.5 mg/mL). *Significantly ($P < 0.05$) different from control.

Treatment ($N = 6$)	Egg diameter (mm)	Fertilization rate (%)[a]	Eye stage (%)
Control	1.12 ± 0.15	98.3 ± 2.06	88.0 ± 3.85
Freshwater	1.44 ± 0.18*	0	
Freshwater and TI	1.42 ± 0.22*	0	
Goldfish Ringer	1.53 ± 0.17*	0	
Goldfish Ringer and TI	1.16 ± 0.10	93.3 ± 5.65	75.3 ± 14.2

[a]In the control group, eggs were fertilized by the traditional dry method immediately after stripping. In the other four treatments, fertilization was tested after 30 min of holding in goldfish Ringer solution or freshwater.

to the eye stage, a level not significantly different from controls (88.0 ± 3.85%).

Discussion

In the present study, the ability of protease inhibitors to block chorion expansion and preserve the fertility of goldfish eggs was assessed. Chorion expansion is a general phenomenon in fish eggs and is presumably related to the maintenance of water balance, prevention of polyspermy (Kobayashi and Yamamoto 1981), and chemical or mechanical protection (Laale 1980; Eddy and Talbot 1985). A significant inhibition of chorion expansion in ovulated eggs was observed in incubates with benzamidine or soybean trypsin inhibitor. These data indicate that expansion of the chorion during fertilization or water activation in goldfish eggs is related to proteolysis. In support of this, it has been shown that proteolysis is required for formation of the zona pellucida in mammals (Hedrick et al. 1987; Moller and Wassarman 1989), formation of the vitelline envelope in sea urchin (Alliegro and Schuel 1988; Somers and Shapiro 1989), and formation of the fertilization envelope in *Xenopus laevis* after fertilization or cortical activation (Gerton and Hedrick 1986; Lindsay and Hedrick 1989). Chorion expansion has also been speculated to involve depolymerization of a major group of glycoproteins (polysialoglycoproteins) which are exocytosed during the cortical reaction in the rainbow trout (*Oncorhynchus mykiss*, formerly *Salmo gairdneri*) (Kitajima and Inoue 1988).

In addition to proteolysis, it has also been shown that an increase in the weight of eggs following activation is inhibited by $AlCl_3$ and $ZnSO_4$ (Eddy and Talbot 1983) and increased salinity (Li et al. 1989) in the Atlantic salmon (*Salmo salar*). The results are attributed to the effects of these chemicals on osmotic influx in the perivitelline space. In the Japanese medaka, Atlantic cod (*Gadus morhua*), and lumpfish (*Cyclopterus lumpus*), extracellular calcium is required for the cortical reaction and subsequent hardening of the chorion (Iuchi et al. 1991; Masuda et al. 1991; Lonning et al. 1984).

Since the precise reaction(s) which was blocked by the protease inhibitors was not investigated in the present study, how chorion expansion and the maintenance of fertility are affected by protease inhibitors is not known. Since eggs treated with protease inhibitor showed no sign of autoactivation and could later be fertilized, it is likely that soybean trypsin inhibitor is acting at a point prior to the closure of the micropyle. In addition, there have been no reports indicating that components of the fish egg envelope are involved in sperm binding as observed in other vertebrates (Wassarman 1987), so it is unlikely that the effect of the soybean trypsin inhibitor on fertility is related to the inhibition of such a process.

The results also indicate that the loss of fertility in goldfish eggs is possibly related to chorion expansion in goldfish Ringer. Even though, as suggested by Szollosi and Billard (1974), the loss of fertility in freshwater or isotonic Ringer's solution might result from the sealing of the internal orifice of the micropyle, the situation might be more complex because activated eggs not only undergo the cortical reaction but also parthenogenetic cleavage.

It is obvious that the presence of soybean trypsin inhibitor is not directly correlated with maintenance of fertility because the eggs treated with soybean trypsin inhibitor in freshwater are not viable. A combination of soybean trypsin inhibitor and a proper osmotic environment was required to maintain fertility of goldfish eggs outside the female.

In the studies on egg fertility reported here, experiments were conducted only to investigate the effects of different treatments at 30 min after the start of incubation. However, preliminary incubations that we have conducted suggest that the eggs can be held for at least 2 h in goldfish Ringer with soybean trypsin inhibitor and retain their fertility.

In summary, we have found that chorion expansion in goldfish eggs can be completely blocked if the proper protease inhibitor is added to a physiological saline solution. Moreover, the eggs held with soybean trypsin inhibitor can later be fertilized with a high rate of success for at least 30 min after stripping. The development of this solution not only provides a means to temporarily hold ovulated goldfish eggs in a viable state outside the female but will also enable and facilitate studies on fertilization and egg cryopreservation in this species.

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Effects of Experimental Manipulations of Light and Phosphorus Supply on Competition among Picoplankton and Nanoplankton in an Oligotrophic Lake

John D. Wehr

Louis Calder Center, Fordham University, Drawer K, Armonk, NY 10504, USA

Wehr, J.D. 1993. Effects of experimental manipulations of light and phosphorus supply on competition among picoplankton and nanoplankton in an oligotrophic lake. Can. J. Fish. Aquat. Sci. 50: 936–945.

Phytoplankton communities of clear, oligotrophic lakes are often dominated by small cells, especially the cyanobacterium *Synechococcus*. Experiments in Rye Lake (New York) directly examined effects of light and phosphorus limitation on the growth of small phytoplankters. Factorial experiments compared growth of phytoplankton collected from 1 and 15 m; microcosms were incubated at both depths and treated with a single P pulse (±2 μM KH_2PO_4). During stratification, picoplankton (0.2–2 μm; ≈*Synechococcus* spp.) predominated, but surface nanoplankton (especially *Cyclotella stelligera* and *Chlamydomonas gloeocapsa*) were stimulated to equivalent biomass levels following the P pulse. No significant P effect was observed at 15 m. Picoplankton biomass was similar in surface and deepwater communities and independent of P treatment. Depletion of PO_4^{3-} was greatest in surface-incubated controls. After turnover, phytoplankton biomass in both size fractions was unaffected by P but decreased when transplanted from 1 to 15 m (−30 to −70%) and increased in transplants from 15 to 1 m (+55 to +140%). Results suggest that (1) *Synechococcus* is a superior competitor only under low-light, low-P conditions, (2) multiple resources interact to affect community size structure, and (3) resultant size shifts significantly alter ecosystem nutrient dynamics.

Les communautés de phytoplancton dans les lacs oligotrophes clairs sont souvent dominées par de petites cellules, et surtout par la cyanobactérie *Synechococcus*. Des expériences ont été effectuées dans le Lac Rye (New York) pour examiner directement les effets de la lumière et du phosphore sur la croissance des petits organismes phytoplanctoniques. Des expériences factorielles ont permis de comparer la croissance du phytoplancton prélevé à une profondeur de 1 et de 15 m; des microcosmes ont été incubés à ces deux profondeurs et traités à l'aide d'une seule impulsion de P (±2 μM de KH_2PO_4). Au cours de la stratification, le picoplancton (0,2 à 2 μm; ≈*Synechococcus* spp.) a prédominé, mais le nanoplancton de surface (surtout *Cyclotella stelligera* et *Chlamydomonas gloeocapsa*) a été stimulé et a atteint des valeurs de biomasse équivalentes après l'impulsion de P. Aucun effet important du P n'a été observé à 15 m. La biomasse du picoplancton était semblable dans les communautés à la surface et en profondeur, et était indépendante du traitement au P. L'épuisement du PO_4^{3-} était la plus importante chez les témoins incubés à la surface. Après renouvellement, la biomasse phytoplanctonique dans les deux fractions de taille n'était pas affectée par le P, mais elle a diminué lorsque les organismes incubés à 1 m ont été transplantés à 15 m (−30 à −70 %) et a augmenté (+55 à +140 %) lorsque la transplantation s'est faite dans le sens inverse (soit de 15 à 1 m). Les résultats indiquent que 1) *Synechococcus* est un compétiteur supérieur seulement lorsque la lumière et la concentration de P sont faibles, 2) les ressources multiples interagissent pour influer sur la structure de taille de la communauté et 3) les modifications de taille qui en résultent modifient de manière importante la dynamiques des éléments nutritifs dans l'écosystème.

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Ecologists usually characterize community structure by the number and proportions of species that occur within an ecologically homogeneous area (Ricklefs 1990). However, descriptions of phytoplankton community structure should consider both the extraordinary species diversity and size range contained within a typical water sample. It is now well established that minute, autotrophic picoplankton (<2 μm) can be an abundant and important part of this community (Stockner 1988) and co-occur with phytoplankters whose cells or colonies exceed 200 or even 2000 μm in size. Expressed in terms of biovolume, the community comprises organisms whose size spans more than five orders of magnitude. Despite these differences, all phytoplankton cells compete for many of the same resources. Therefore, a study of factors that regulate phytoplankton communities should consider the size structure as well as the species structure of these assemblages.

Resource competition is important in structuring phytoplankton communities, and experiments under steady-state (chemostat) conditions have shown that competition for two limiting resources can affect the species composition of freshwater communities (see reviews by Kilham and Hecky 1988; Sommer 1989). The demonstration of interactions among limiting resources in non-steady-state (pulsed) systems, such as lakes, is still needed. The latter approach, while less controlled, is more realistic and allows more subdominant species to co-occur (Sommer 1985). A pulse of P can result in the increase of a certain phytoplankton species, which may intensify competition for light. Changes in light availability cause cells to expe-

rience a nonsteady supply of another limiting resource, even in "steady-state" experiments. Changes in light availability, light quality, and light–dark cycles favor certain species and alter community structure (Wall and Briand 1979; Chisholm 1981; Glover et al. 1986; Lewis et al. 1986). However, interactions between light availability and nutrient limitation have rarely been tested by direct experimental manipulation (Ward and Wetzel 1980).

The dominance of small phytoplankton species in certain lakes is thought to be the outcome of competition for limiting nutrients. Correlative and experimental data indicate that P limitation (e.g., high N:P ratios) favors smaller cells in freshwaters, particularly species of the cyanobacterium *Synechococcus* (Suttle et al. 1987; Stockner and Shortreed 1988; Watson and McCauley 1988; Wehr 1989). At P levels >0.2 μM, the size spectrum usually shifts toward larger cells (Stockner and Shortreed 1988; Wehr 1989). This balance among cell sizes may also be affected by light, as well as nutrients. In order to resolve whether resource competition is important in any community, one must determine both the effect of resource limitation and the intensity of resource depletion (Goldberg 1990). Among phytoplankton species, the mechanism behind different patterns in size structure is apparently a greater rate of inorganic PO_4^{3-} uptake in smaller cells during P-limited conditions (Lean and White 1983; Suttle and Harrison 1988).

In this paper, I test whether two key resources, P supply and light availability, interactively limit the growth of phytoplankton and affect community structure in an oligotrophic lake. Experiments are run in situ with whole phytoplankton communities; treatments are single pulses of inorganic P and irradiance shifts, using incubations at two depths. Treatments are applied in a combined, or factorial, manner in order to assess the separate and interactive effects of two important resource gradients on the phytoplankton community. I evaluate effects of competition in terms of changes in (1) size structure, (2) species composition, and (3) resource depletion.

Materials and Methods

Study Site

Experiments were carried out in Rye Lake, a moderately deep lake located in southeastern New York State (41°04′N, 73°44′W). The lake basin was connected to Kensico Lake in the early 1900's with the formation of Kensico Reservoir (890 ha, $Z_{max} \approx 50$ m). The surface area of Rye Lake itself is ~260 ha and maximum depth is ~40 m. The system lies within a crystalline, gneiss–schist bedrock and levels of soluble reactive P (SRP) and soluble total P are consistently low (<0.05 and 0.25 μM, respectively). The basin stratifies in spring, and autumn turnover usually occurs in late October or November. Other physical and chemical features of the Kensico system were described previously (Wehr 1991). Samples were processed at the laboratories of the Louis Calder Center, Fordham University (Armonk, NY), located ~8 km from the study site.

Field Procedures and Experimental Design

Experiments were run on two 4-d periods, 23–27 September (during stratification) and 2–6 November (after turnover) 1990. Subsequent physical and chemical analysis revealed that on the November dates, temperatures were isothermal (eliminating temperature effects in experimental treatments), but chemical conditions were not uniform with depth. For the purposes of this paper, the term "after turnover" will be retained. On each starting date, a triplicate set of water chemistry and phytoplankton samples were collected from 1 and 15 m, and temperature and light profiles were measured. A set of phytoplankton samples for size-fractionated biomass estimates was also collected and measured. The methods used were the same as outlined below for experiment harvest periods. Light was measured using a Li-Cor (LI-185A) PAR meter fitted with a submersible cosine probe, and temperature was recorded using a YSI temperature/O_2 meter.

A three-way factorial design was used in which community depth (1 or 15 m), incubation depth (1 or 15 m), and a single P pulse (0 or +2 μM) were factor treatments (a total of eight treatments × three replicates). The intent of this design was to examine the separate and interacting effects of depth, light, and nutrients on the size structure and competition among phytoplankton in a clearwater, oligotrophic system. The lake is P limited, with relatively high levels of inorganic N (~30 μM NO_3, 0.5 μM NH_4; see also Wehr 1991). Effects of these manipulations on nutrient (PO_4, total P, NO_3, NH_4) depletion were also measured.

Water was collected with a peristaltic pump from the 1- and 15-m depths (~90 and 1% surface irradiance; labeled I_0) into triplicate 4-L containers, which served as experimental microcosms. Prior to each experiment, larger zooplankton were removed using 202-μm Nitex mesh (to minimize container-exaggerated herbivory effects). Microcosms were attached to an anchored float near the deepest part of Rye Lake. Half the microcosms were fertilized with a single pulse of KH_2PO_4 (stock[P] = 4 mM) using syringes fitted with 0.2-μm pore size sterile filter units (Gelman acrodisc) to a final concentration of 2 μM P. They were then fully mixed and attached to the floats. Bags were positioned at either 0.5 or 14 m, so that light penetration in the bags approximated levels measured in situ at the 1- and 15-m depths, respectively.

After 4 d of incubation, bags were removed and phytoplankton were collected with a pump fitted with an in-line filter holder containing 20-μm Nitex mesh. Filters and the remaining filtrate were kept cold (4°C) and dark until returned to the laboratory. Water collected for nutrient analysis and phytoplankton identification was pumped without prefiltration. Nutrient samples were immediately postfiltered (0.45-μm pore size), stored cold on the boat, and frozen within 3 h of collection.

Laboratory Procedures

Size categories of phytoplankton follow earlier definitions (Sieburth et al. 1978; Wehr 1989). Briefly, microplankton are cells or colonies 20–200 μm, nanoplankton are 2–20 μm, and picoplankton are 0.2–2 μm. The filtering methods (including care to prevent disruption of more fragile organisms) have been described previously (Wehr 1989, 1990). Chlorophyll *a* (Chl *a*) was extracted by placing filters in vials containing 3–4 mL of neutral 90% acetone (with $MgCO_3$), grinding with a metal spatula, and extracting cold (<4°C) in the dark for 18–24 h. Extracts were made up to 5 mL and centrifuged; Chl *a* was then measured spectrophotometrically (Shimadzu model UV-160) after correction for pheophytin *a* (Lorenzen 1967; APHA 1985).

Whole phytoplankton samples were split in half; one aliquot was preserved with Lugol's iodine and the other was preserved with glutaraldehyde to final concentration of 2% and filtered onto 25-mm black-stained Poretics filters (0.2-μm pore size).

TABLE 1. Physical and chemical conditions at the surface (1 m) and in deeper water (15 m) in Rye Lake at the start of each depth–phosphorus experiment. Stratification period sampled on 23 September 1990; post–turnover sampling on 2 November 1990 (irradiance in μmol photons·m^{-2}·s^{-1}; depth of 1% light level in m, alkalinity in mEq/L; SRP = soluble-reactive phosphorus; nutrient concentrations in μM).

	During stratification		After turnover	
Variable	1 m	15 m	1 m	15 m
Temperature (°C)	18.2	12.8	13.2	12.5
Irradiance	270	2.3	42	0.18
Weather conditions	Sunny and calm		Overcast and windy	
1% light level	14.1		10.5	
pH	7.4	7.6	7.9	7.5
Alkalinity	0.23	0.20	0.24	0.24
SRP	0.07	0.05	0.04	<0.03
Total P	0.26	0.19	0.29	0.23
NO_2^-	1.1	0.91	0.61	0.99
NO_3^-	19.6	37.7	31.1	32.6
NH_4^+	0.32	0.85	0.78	0.49

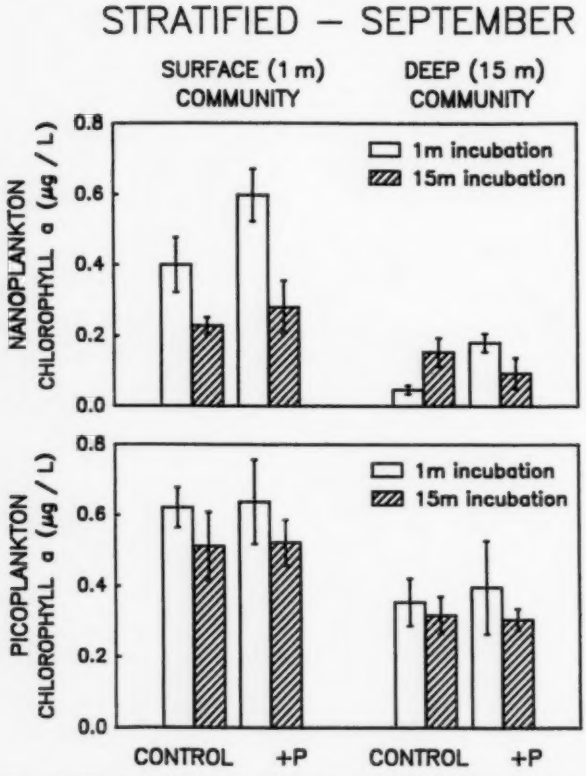

FIG. 1. Effects of surface (1 m) and deep (15 m) incubation and a single 2 μM PO_4^{3-} pulse on the biomass of autotrophic nanoplankton (cells 2–20 μm) and picoplankton (cells 0.2–2 μm) collected from 1 and 15 m during stratification. Biomass measured as Chl *a* densities in 4-L microcosms after 4 d of incubation (open bars = 1-m incubation; shaded bars = 15 m incubation; error bars indicate ±1 SE; $n = 3$).

TABLE 2. Summary of three-way ANOVA testing the combined and interacting effects of community depth, incubation depth, and P addition on the biomass (measured as Chl *a*) of algal nanoplankton and picoplankton in Rye Lake during stratification. The experiment was run 23–27 September 1990 ($n = 3$); F score; P = probability).

Response factor	F	P
Nanoplankton		
Community depth	52.45	<0.001***
Incubation depth	10.73	0.005**
P addition	5.17	0.037*
Community × incubation	12.58	0.003**
Community × P addition	1.55	0.231 NS
Incubation × P addition	5.69	0.030*
Community × incubation × P addition	0.12	0.734 NS
Picoplankton		
Community depth	14.98	0.001**
Incubation depth	2.23	0.155 NS
P addition	0.05	0.819 NS
Community × incubation	0.17	0.685 NS
Community × P addition	0.01	0.989 NS
Incubation × P addition	0.06	0.806 NS
Community × incubation × P addition	0.04	0.849 NS

Phytoplankton (with the exception of autotrophic picoplankton) were counted and identified using the sedimentation and inverted microscope method (Utermöhl 1958) with a Nikon Diaphot inverted microscope. Random comparisons with epifluorescence counts found that the Utermöhl technique underestimated numbers of picoplankton cells by about 50%. Epifluorescent counts of autotrophic picoplankton cells followed methods described elsewhere (Caron et al. 1985; Hawley and Whitton 1991), using a Nikon Labophot microscope with separate blue (EX 450–490; DM 510; BA 520) and green (EX 500–550; DM 575; BA 580) filter sets. Only yellow–orange fluorescing cells were observed, so that the blue filter set was used for routine counts. For each method, a minimum of 10 grids and 1000 cells were counted per sample replicate. Counts were converted to biovolume (average of ≥20 cell measurements/species) using geometric formulas given in Wetzel and Likens (1991).

Water samples were analyzed for SRP via the antimony–ascorbate–molybdate method (APHA 1985; Bran+Luebbe

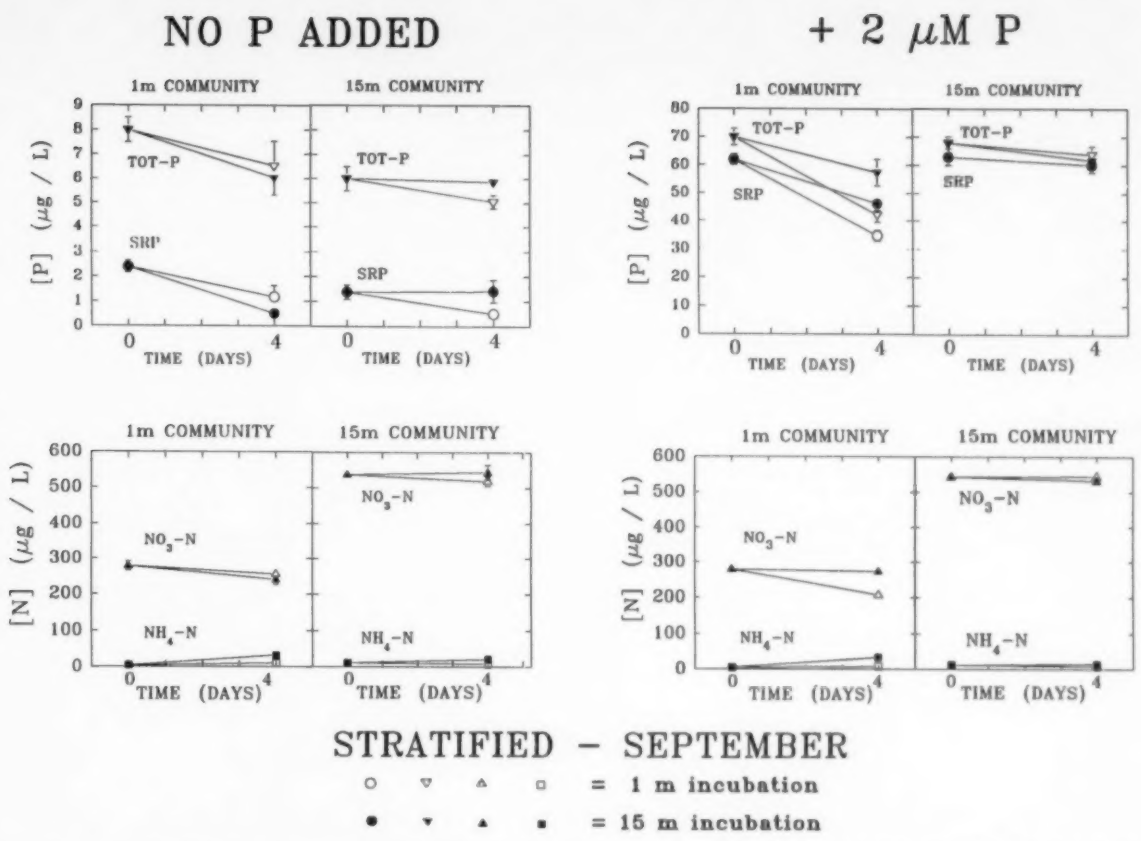

FIG. 2. Effects of surface (1 m, open symbols) and deep (15 m, solid symbols) incubation on 4-d depletion of PO_4^{3-} (SRP), total dissolved P (TOT-P), NH_4^+, and NO_3^- from stratified surface and deep water (23–27 September 1990). Data are plotted according to community and incubation depth and paired for control and P-pulsed microcosms (open symbols = 1-m incubation; solid symbols = 15-m incubation; error bars indicate ±1 SE; n = 3).

Analyzing Technologies, Inc. 1986a). Total dissolved P was determined in an identical manner, following persulfate digestion (Menzel and Corwin 1965). NH_4^+-N was determined via the phenol–hypochlorite method, and NO_3^- was determined using sulfanilamide–NNED, following reduction to NO_2^- in a Cd–Cu column (APHA 1985; Bran + Luebbe Analyzing Technologies, Inc. 1986b, 1987). Methods were modified for automated analysis and run on a Traacs 800 automated analyzer (Bran + Luebbe Analyzing Technologies, Inc.).

Data were compiled and analyzed using the SYSTAT 4.2 statistical program (Wilkinson 1988). Hypothesis tests were set up as model I ANOVA analyses with factors A (community depth), B (incubation depth), and C (P addition) as independent variables. Tests of factor interactions and selected comparisons of means were also performed using ANOVA for individual t-tests. Effects were judged to be significant if the probability of an event occurring due to chance (P) was <0.05.

Results

Lake Conditions Before and After Stratification

The two experimental periods represented distinct physical conditions. There was more than a 5°C vertical thermal gradient during September and isothermal, cooler conditions in November (Table 1). During the first exepriment, light availability and water clarity were greater (1% surface irradiance at 14.1 m) than during fall mixis (1% level at 10.5 m). Chemical conditions changed slightly, as turnover resulted in an increase in only surface NO_3^-. Total dissolved inorganic N (DIN) concentrations always exceeded 20 μM. SRP remained at or below the detection limit (0.03 μM, ~1 μg/L) despite the turnover, while total dissolved P ranged between 0.2 and 0.3 μM (~8–9 μg/L), with no apparent differences with respect to depth or mixing. Other chemical variables (e.g., pH, alkalinity) were stable between experimental dates.

Experiment 1: During Stratification

Total phytoplankton biomass, as estimated by Chl *a* concentrations, was ~1.4 μg/L at the surface and ~0.64 μg/L at 15 m (below the thermocline). The microplankton size fraction (>20 μm) represented the smallest proportion (≤20%) of this total. On average, algal nanoplankton (>2–20 μm) plus picoplankton (<2 μm) combined to total more than 75% of phytoplankton biomass. Surface communities in situ had significantly greater biomass (as Chl *a*) than those from 15 m. Incubation of surface communities at this lower light level (~1% surface irradiance) resulted in a significant reduction

TABLE 3. Effects of a single P addition and changing light field (via incubation depth) on the biovolume (μm^3/mL) of the 10 most common phytoplankton species (>80% total biovolume) found in Rye Lake. Experiment 1 was conducted 23–27 September 1990 during stratification (values are means and standard errors from three replicate bags; P = phosphorus effect significant at $p < 0.05$; D = community depth effect significant at $P < 0.05$; I = incubation depth effect significant at $P < 0.05$).

	Biovolume during stratification			
	No P added Community depth (m)		+2 μM P Community depth (m)	
Species	1	15	1	15
Incubation depth = 1 m				
Cyanobacteria				
Aphanothece clathrata	68 ± 16	34 ± 8	82 ± 30	22 ± 17 D
Synechococcus spp. (<2 μm)	1022 ± 44	1001 ± 112	1043 ± 24	1080 ± 74
Chlorophyta				
Chlamydomonas gloeocapsa	142 ± 15	62 ± 24 D	383 ± 46 P	44 ± 18 D
Chrysophyta				
Dinobryon cf. *cylindricum*	70 ± 10	82 ± 17	112 ± 28	<8 I
Ochromonas sp. (~3–4 μm)	127 ± 28	12 ± 2 D	177 ± 25	36 ± 2
Bacillariophyta				
Asterionella formosa	<20	<20	37 ± 18 P	<20
Cyclotella stelligera	119 ± 24	60 ± 24 D	1237 ± 113 P	71 ± 24 D
Fragilaria crotonensis	95 ± 29	48 ± 14	190 ± 63	142 ± 48
Rhizosolenia longiseta	302 ± 65	<29	388 ± 155	129 ± 35
Pyrrophyta				
Peridinium pusillum	54 ± 12	<32 D	<32	<32
Incubation depth = 15 m				
Cyanobacteria				
Aphanothece clathrata	20 ± 6 I	36 ± 11	39 ± 15	39 ± 3
Synechococcus spp. (<2 μm)	1039 ± 70	1013 ± 51	1154 ± 276	1094 ± 335
Chlorophyta				
Chlamydomonas gloeocapsa	55 ± 26 I	70 ± 15	66 ± 9 I	11 ± 9 D
Chrysophyta				
Dinobryon cf. *cylindricum*	56 ± 25	70 ± 25	168 ± 82	28 ± 13 D
Ochromonas sp. (~3–4 μm)	50 ± 7 I	17 ± 5	70 ± 23 I	37 ± 9
Bacillariophyta				
Asterionella formosa	<20	<20	<20	<20
Cyclotella stelligera	71 ± 12 I	119 ± 34	250 ± 70 I, P	48 ± 12
Fragilaria crotonensis	48 ± 6	<32	48 ± 16	<32 I
Rhizosolenia longiseta	216 ± 25	43 ± 25	259 ± 80	<28 I
Pyrrophyta				
Peridinium pusillum	<32	<32	<32	<32

(40–60%, $P < 0.01$) in nanoplankton biomass, yet the biomass of algal picoplankton was unaffected (Fig. 1). A single 2-μM pulse of P stimulated nanoplankton biomass ($P < 0.05$), but only when incubated at the surface, and regardless of whether the community originated from 1 or 15 m. A 2-μM P pulse had no significant effect on biomass of algal picoplankton. Picoplankton biomass was less at 15 m than at the surface, but incubation of the deeper community at greater light levels did not promote an increase in measured Chl *a*.

ANOVA indicates that the only significant difference in picoplankton among all treatments was community depth (1 versus 15 m) and that none of these experimental treatments significantly interact to affect biomass (Table 2). Nanoplankton biomass was affected by all three treatments, and depth of incubation significantly altered responses to P enrichment. The effect of these size shifts was that the P-enriched communities with sufficient light had similar Chl *a* levels in the nanoplankton (0.60 ± 0.06 μg/L) and picoplankton (0.63 ± 0.07 μg/L), while either low light and/or low P (ambient) favored the maintenance of picoplankton-dominated assemblages (pico > nano in all paired comparisons, $P < 0.05$).

Changes in the biomass and size structure were reflected in community-level depletion of nutrients over the 4-d period (Fig. 2). SRP and total P were depleted most rapidly in the enclosures that contained surface communities. This effect was most noticeable in surface communities with 2 μM P added, where this change averaged −46% when incubated at 1 m and −27% when incubated at 15 m (depth effect significant, $P < 0.05$). Changes in total dissolved P parallel this latter decrease. Greater P depletion corresponded to a significant increase in nanoplankton Chl *a*. NH_4^+ concentrations were relatively low (<0.4 μM) throughout the experiment. Incubation of surface waters at 15 m resulted in greater measured NH_4^+ (+10–20 μg/L, ≈+0.7–1.4 μM), with a corresponding (roughly equivalent) decrease in NO_3^-. Thus, no net changes in DIN were observed.

The phytoplankton community during late summer was predominantly composed (based on cell numbers and biovolume) of small blue-green cells (attributed to *Synechococcus* spp.) and lesser biovolumes of flagellates (*Ochromonas* sp., *Chlamydomonas gloeocapsa*) and a small centric diatom, *Cyclotella stelligera* (Table 3). The latter three phytoplankters all fall within

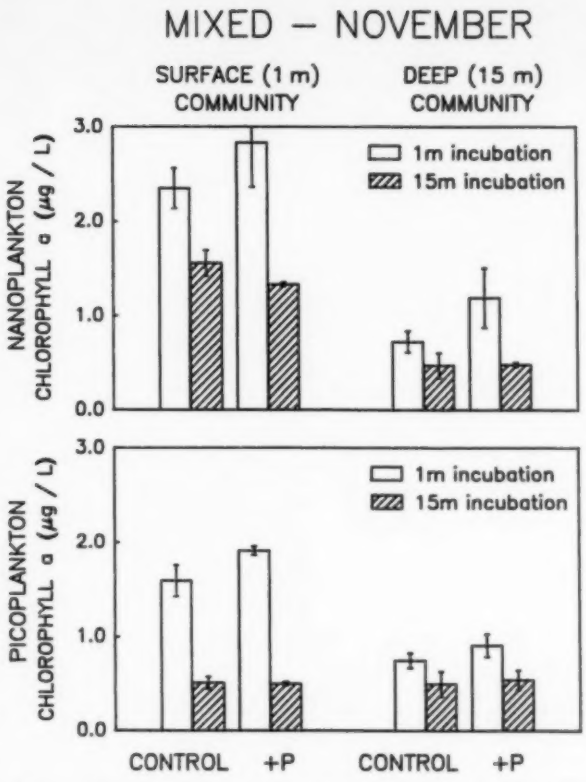

FIG. 3. Effects of surface (1 m) and deep (15 m) incubation and a single 2 μM PO_4^{3-} pulse on the biomass of autotrophic nanoplankton (cells 2–20 μm) and picoplankton (cells 0.2–2 μm) collected from 1 and 15 m following turnover (further details as in Fig. 1).

the nanoplankton size range (2–20 μm maximum dimension). Transplanting nanoplankters from surface (1 m) to deeper water (15 m) resulted in reduced growth rates and biomass (see "I" symbol in Table 3) of several taxa, including *Aphanothece clathrata*, *C. gloeocapsa*, *Cylcotella stelligera*, and *Ochromonas* sp. These effects of light limitation were most extreme in enclosures receiving a 2 μM P pulse. For example, *C. gloeocapsa* and *C. stelligera* biovolumes each decline about 80%. Cell numbers of *Synechococcus* spp. (range 3.1×10^5 to 3.6×10^5/mL) were not significantly different among the eight treatments. The P pulse shifted the species composition at the surface from a *Synechococcus*-dominated community (51% of total biovolume) to a more diverse *Cyclotella–Chlamydomonas–Ochromonas* community. Because there was no absolute effect of P addition on picoplankton biomass, *Synechococcus* spp. relative biovolume levels dropped to about 20% of the total.

Experiment 2: After Turnover

Mixing was presumed to eliminate possible confounding effects of temperature on light/depth treatments, although concentrations of total inorganic N roughly doubled in surface waters (Table 1). Total algal biomass at the surface increased from 1.4 to 4.5 μg Chl *a*/L and nanoplankton biomass (~52% of the total) was significantly greater than picoplankton (~35%, $P < 0.01$; Fig. 3). There were no strong responses to a 2 μM P pulse during this period, despite similar total P levels (~8 μg/L in September versus ~9 μg/L in November). There was broad evidence for light limitation in most organisms at 15 m. Surface nanoplankton and picoplankton had reduced biomass when incubated at lower light (temperatures equal). Deeper communities had similar biomass levels of nano- and picoplankton and experienced small but significant increases when incubated near the surface. ANOVA supports this result (Table 4); greater depth significantly reduced the biomass in both size fractions, but in November, this effect was apparently greatest in the picoplankton (−70 to −75%, $F = 134.98$).

TABLE 4. Summary of three-way ANOVA testing the combined and interacting effects of community depth, incubation depth, and P addition on the biomass (measured as Chl *a*) of algal nanoplankton and picoplankton in Rye Lake following turnover (isothermal conditions, see Materials and Methods). The experiment was run 2–6 November 1991 ($n = 3$); $F = F$ score; P = probability).

Response factor	F	P
Nanoplankton		
Community depth	65.72	<0.001***
Incubation depth	25.72	0.001***
P addition	1.31	0.269 NS
Community × incubation	4.37	0.053 NS
Community × P addition	0.11	0.744 NS
Incubation × P addition	3.25	0.090 NS
Community × incubation × P addition	0.15	0.700 NS
Picoplankton		
Community depth	44.19	<0.001***
Incubation depth	134.98	<0.001***
P addition	3.62	0.075 NS
Community × incubation	45.90	<0.001***
Community × P addition	0.01	0.953 NS
Incubation × P addition	2.57	0.128 NS
Community × incubation × P addition	1.27	0.276 NS

The two experiments demonstrate how the same experimental treatments may have different effects over time. Turnover led to a different assemblage with greater total biomass and a shift from picoplankton to nanoplankton dominance at the surface. In deeper waters, picoplankton biomass remained at levels similar to those during summer stratification; biomass of larger nanoplankton cells increased two- to threefold ($P < 0.01$). Changes in water chemistry due to turnover were seen in total inorganic N, but not in total P (Table 1). Few experimental treatments resulted in a significant depletion of nutrients over 4 d (Fig. 4). Following turnover, P-enriched enclosures (dominated by nanoplankters) did not experience large declines in SRP over time, although total P was reduced in surface communities. Following incubation at 15 m, decreases in NH_4^+ and increases in NO_3^- were again observed, although they were less than the levels during stratification.

The phytoplankton community was more evenly composed of cyanobacteria, chrysophytes, dinoflagellates, and diatoms. The most important taxa were *Synechococcus* spp., *Fragilaria crotonensis*, *Ochromonas* sp., and *Peridinium pusillum* (Table 5). Some larger diatoms, including *Rhizosolenia eriensis*, increased in importance, although total microplankton Chl *a* (0.3–0.6 μ/L, 14–22% of the total) and biovolume

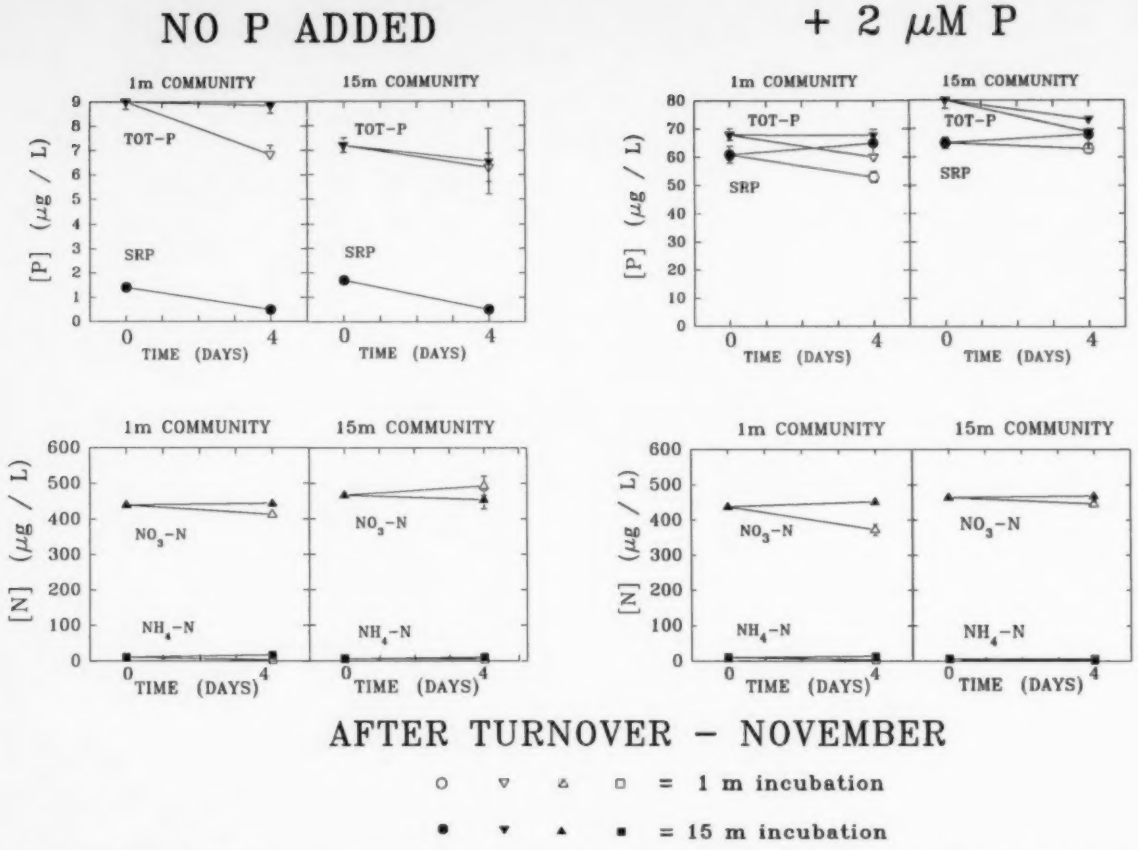

FIG. 4. Effects of surface (1 m, open symbols) and deep (15 m, closed symbols) incubation on 4-d depletion of PO_4^{3-} (SRP), total dissolved P (TOT-P), NH_4^+, and NO_3^- from surface and deep water following turnover (2–6 November 1990) (further details as in Fig. 2).

(<900 μm³/mL, ~20% of the total) remained low. Following mixis, there were few effects of P addition on biovolume levels of any phytoplankton species, except *F. crotonensis*. Most major species, excluding *Synechococcus* spp., declined in response to downward transplant, and nearly all increased significantly when moved from 15 to 1 m. Light limitation was most pronounced in the chrysophytes, diatoms, and dinoflagellates in enclosures with +2 μM P. For example, decreased light resulted in roughly a 90% decline in the biovolume levels in *Ochromonas* sp. and a 55–70% decline in *P. pusillum*. Overall, low light favored picoplankton due to the strong negative effects on larger phytoplankton species.

Discussion

Competitive interactions among large and small phytoplankton cells for scarce resources depend on a complex of other factors that affect the community and the rate and frequency at which these resources become available. For example, studies have shown that increasing P limitation in freshwaters favors a predominance of smaller cells, such as *Synechococcus* spp. (Suttle and Harrison 1988; Wehr 1989). However, an increase in the frequency of limiting nutrient additions ("pulses") may favor larger cells (Turpin and Harrison 1979; Suttle et al. 1987, 1988). Such studies emphasize the importance of not only the level of a nutrient addition, but also its timing and how a pulse may interact with other factors. A single, large (2 μM) pulse of PO_4^{3-} to oligotrophic Rye Lake had a variable effect on the phytoplankton species present and on rates of nutrient depletion. The Rye Lake experiments support earlier studies that indicate that elevated P levels favor larger cells but also show that such treatments interact significantly with other key resources. These interactions are also subject to change in response to seasonally varying conditions within a lake, such as light, temperature, and mixing conditions.

Individual species responses in Rye Lake also support earlier findings. In Rye Lake, the diatom *C. stelligera* proved to be a poor competitor for PO_4^{3-}, as has been shown for a related and similar-sized *C. meneghiniana* (Kilham and Hecky 1988). However, at low SRP levels, densities of putative strong competitors for $PO^3{}_4^-$, such as *Asterionella formosa*, remained low (perhaps due to low Si levels, <2 mg/L). The abundance of *Synechococcus* spp. in Rye Lake (~2.4×10^5 to 3.2×10^5 cells/mL) is similar to that observed in other oligotrophic systems, such as Lake Ontario (Caron et al. 1985), Kennedy and Sproat lakes, British Columbia (Suttle et al. 1988), and Lake Superior (Fahnenstiel et al. 1986), but exceeds densities recorded for oligotrophic, but more calcareous Lakes Huron and Michigan (Fahnenstiel and Carrick 1992). An increase in the relative importance of larger cells in Rye Lake, especially

TABLE 5. Effects of a single P addition and changing light field (via incubation depth) on the biovolume (μm^3/mL) of the 10 most common phytoplankton species (>80% total biovolume) found in Rye Lake. Experiment 2 was conducted 2–6 November 1990 following turnover (values are means and standard errors for three replicate bags; P = phosphorus effect significant at $p < 0.05$; D = community depth effect significant at $P < 0.05$; I = incubation depth effect significant at $P < 0.05$).

	Biovolume after turnover			
	No P added Community depth (m)		+2 μM P Community depth (m)	
Species	1	15	1	15
Incubation depth = 1 m				
Cyanobacteria				
Aphanothece clathrata	3 ± 0.8	<0.4	3 ± 1.2	<0.4
Synechococcus spp. (<2 μm)	780 ± 35	614 ± 115	736 ± 33	632 ± 43
Chlorophyta				
Chlamydomonas gloeocapsa	58 ± 14	24 ± 14 D	66 ± 13	22 ± 7 D
Chrysophyta				
Dinobryon cf. *cylindricum*	328 ± 28	63 ± 8 D	370 ± 54	77 ± 19 D
Ochromonas sp. (~3–4 μm)	669 ± 87	227 ± 38 D	681 ± 56	155 ± 38
Bacillariophyta				
Asterionella formosa	113 ± 25	225 ± 22	112 ± 23	187 ± 36
Cyclotella stelligera	48 ± 16	143 ± 16	48 ± 22	75 ± 19
Fragilaria crotonensis	666 ± 120 I	1023 ± 170	1094 ± 213 P	761 ± 189
Rhizosolenia longiseta	216 ± 43	86 ± 11 D	86 ± 28	65 ± 44
Pyrrophyta				
Peridinium pusillum	539 ± 63	27 ± 16 D	485 ± 32	18 ± 16 D
Incubation depth = 15 m				
Cyanobacteria				
Aphanothece clathrata	<1	3.2 ± 0.8	<1	2.4 ± 0.8
Synechococcus spp. (<2 μm)	677 ± 46	821 ± 41	867 ± 162	792 ± 143
Chlorophyta				
Chlamydomonas gloeocapsa	88 ± 7	44 ± 21 D	66 ± 13	88 ± 33
Chrysophyta				
Dinobryon cf. *cylindricum*	419 ± 62	98 ± 21	344 ± 70	133 ± 56 D
Ochromonas sp. (~3–4 μm)	50 ± 8 I	208 ± 36	81 ± 16 I, P	134 ± 19
Bacillariophyta				
Asterionella formosa	75 ± 22	19 ± 15	<6 I	<6
Cyclotella stelligera	143 ± 32	129 ± 18	40 ± 16	32 ± 12
Fragilaria crotonensis	1237 ± 252	238 ± 110	262 ± 51 I	119 ± 30 D
Rhizosolenia longiseta	151 ± 29	43 ± 37 D	86 ± 22	43 ± 14 D
Pyrrophyta				
Peridinium pusillum	243 ± 41 I	54 ± 27 D	135 ± 13 I	27 ± 16 D

the nanoplankters *Ochromonas* sp. and *P. pusillum* and the larger diatom *F. crotonensis*, following turnover, suggests that mixis may be an important factor affecting size structure, through changes in either nutrient or light conditions.

It has been suggested that phycoerythrin-rich cyanobacteria are adapted to reduced light fields (<5% I_0) and may predominate at greater depths (Glover 1985; Lewis et al. 1986). More recent data from the Sargasso Sea suggest that other picoplankton cells (eucaryotic algae) may be equally important (Glover et al. 1988). Reduced light fields in Rye Lake favored *Synechococcus* spp. during stratification, primarily as a result of a declining biomass in several nanoplankton species. This interaction was less pronounced, however, after autumn mixis, presumably because decreased water clarity resulted in more intense light limitation (~0.2% I_0) at 15 m depth. There was an important difference in the apparent responses of picoplankton to reduced light, following turnover. Biovolume data indicate that *Synechococcus* cell densities were *not* significantly reduced, but Chl *a* data suggest a large and significant inhibition. The results highlight the fact that biomass estimates by these two methods may not always agree. Under very low photon flux (<1% I_0), numbes of *Synechococcus* cells may not have changed within 4 d, while cell-specific chlorophyll levels declined. Such cells may then depend more strongly on the light-harvesting abilities of phycoerythrin complexes (Lewis et al. 1986). Alternatively, this discrepancy may be due to inefficient filtration of picoplankton cells for chlorophyll analysis (2-μm filters may trap small cells when collecting nanoplankton).

ANOVA revealed more subtle effects in these competitive relationships. Strong P limitation of nanoplankton during stratification at greater incubation depth meant that light limitation became more severe following a 2 μM PO_4^{3-} pulse (judged from a significant incubation × P addition interaction). Competition among phytoplankton for light in deeper strata of oligotrophic lakes may be intensified by occasional pulses of nutrients.

Few studies have tested light limitation among picoplankton and larger phytoplankton in situ. Wall and Briand (1979) examined the effects of reduced irradiance and altered light quality on phytoplankton species composition in Heney Lake, Quebec. More than 75% of Heney Lake species showed no response to

experimental manipulations, but certain species of green algae, colonial cyanobacteria, and diatoms were favored under "high-light" conditions (total irradiance >5% I_0). Two taxa (*Peridinium* sp., *Gomphosphaeria lacustris*) were competitively favored at reduced light (<5% I_0). It was not clear how nutrient levels may have affected the results at Heney Lake or whether smaller coccoid cyanobacteria were important. The most frequently occurring species in Heney were large, colonial, heterocystous cyanobacteria; systems typified by these organisms tend to have low densities of picoplankton (Watson and McCauley 1988; Wehr 1991). In Rye Lake, light limitation was at times a function of nutrient availability. Competition for light may only proceed in oligotrophic lakes following pulses of limiting nutrients or following holomixis. Given the demonstrated importance of light limitation to many phytoplankters and the interactive effects that can result following nutrient pulses, it should be recognized in future studies that the alleviation of one limiting resource (e.g., PO_4^{3-}) may induce competition for a secondary resource. Autotrophic picoplankton, and in particular PE-rich *Synechococcus* spp., may outcompete larger cells at greater depths in clear, oligotrophic lakes because these cells are less affected by P limitation than other species (e.g., Suttle and Harrison 1988; Wehr 1989) but also because of greater competitive abilities under low light levels. This may be particularly important during brief periods of nutrient pulses when an increase in the numbers of larger phytoplankton cells reduces water clarity. *Synechococcus* spp. biovolume and total picoplankton biomass (as Chl *a*) were unaffected by cross-transplants between the 1- and 15-m depths during late summer, suggesting that irradiance levels approaching 1% I_0 may be sufficient to support autotrophic picoplankton in oligotrophic lakes.

Interacting effects of light and P limitation can also have indirect effects on nutrient cycling and turnover. In Rye Lake, reduced irradiance (incubation at 15 m) gives *Synechococcus* a competitive advantage. This resulted in increased rates of P depletion and apparent regeneration of NH_4^+. Following a 2 μM PO_4^{3-} pulse, nanoplankter species predominate, and P depletion rates decline. Size structure of a phytoplankton community can have dramatic effects on nutrient depletion and cycling (Lean and White 1983). In oligotrophic Flathead Lake (Montana), experimental data suggest that pelagic microorganisms dominate uptake and regeneration of NH_4^+ and PO_4^{3-} during stratification (Dodds et al. 1991). Recognition of the factors that regulate the competitive interactions among microbial species therefore is important for understanding nutrient dynamics in lakes. In Rye Lake, there is evidence for a kind of microbial cascade in which competition for light and/or P initiates a shift towards smaller cells. This in turn alters the rates of cycling and turnover of key nutrients within the water column.

Acknowledgements

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A Novel Approach to Evaluate Feeding by Mixed Cyprinid Species in a Chinese Integrated Fish Culture Pond Using Measurements of Chlorophyll Derivatives and Photosynthesis in Gut Contents[1]

Noriko Takamura

Environmental Biology Division, National Institute for Environmental Studies, 16-2 Onogawa, Tsukuba, 305 Japan

Ja-Le Li, He-Quan Yang, and Xue-Bao Zhu

Shanghai Fisheries University, 334 Jungong Road, Shanghai 200090, P.R. China

and Taizo Miura[2]

Center for Ecological Research, Kyoto University, Shimosakamoto, Otsu, 520-01 Japan

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Chlorophyll derivatives and algal photosynthesis in the guts of five reared cyprinid species were studied to quantify feeding by cyprinids in a Chinese integrated fish culture pond. Pigment analysis identified organic detritus that had been overlooked by microscopic observation. The high ratios of Chl *b*/Chl *a* and of pheophorbide *a*/Chl *a* were good indicators of herbivory on aquatic plants and zooplanktivory, respectively. High photosynthetic rates in faeces indicated herbivory on phytoplankton. Grass carp (*Ctenopharyngodon idella*) fed mostly on aquatic plants. Blunt snout bream (*Megalobrama amblyocephala*) selected plants, but also fed on filamentous algae and zooplankton. Silver carp (*Hypophthalmichthys molitrix*) fed on phytoplankton most frequently among the five carp species examined, but also utilized zooplankton and amorphous detritus, probably originating from plant fragments. Bighead (*Aristichthys nobilis*) and crucian carp (*Carassius auratus*) utilized zooplankton and plant fragments. Planktivorous production in this pond was partly supported by a large amount of plant debris, derived from the faeces of grass-eating cyprinids and nourished by microorganisms in the pond water.

Nous avons étudié les dérivés de la chlorophylle et la photosynthèse algale dans les intestins de cinq espèces de cyprinidés d'élevage pour quantifier l'alimentation de ces espèces dans un système intégré de pisciculture en Chine. L'analyse des pigments a révélé la présence de détritus organiques qui n'avaient pas été vus au microscope. Les rapports élevés de la Chl *b*/Chl *a* et du phéophorbide *a*/Chl *a* étaient de bons indicateurs respectivement de l'alimentation des herbivores (plantes aquatiques) et des zooplanctivores. Un taux élevé de photosynthèse dans les fèces indiquait que les herbivores se nourrissaient de phytoplancton. La carpe de roseau (*Ctenopharyngodon idella*) se nourrissait surtout de plantes aquatiques. La brème (*Megalobrama amblyocephala*) herbivore privilégiait les plantes, mais se nourrissait aussi d'algues filamenteuses et de zooplancton. Parmi les cinq espèces examinées, la carpe argentée (*Hypophthalmichthys molitrix*) était celle qui se nourrissait le plus fréquemment de phytoplancton, mais elle absorbait aussi du zooplancton et des détritus amorphes provenant probablement de fragments végétaux. La carpe marbrée (*Aristichthys nobilis*) et le cyprin (*Carassius auratus*) consommaient du zooplancton et des fragments de plantes. Dans l'étang, la production des planctivores était soutenue en partie par une grande quantité de débris végétaux, provenant des fèces des cyprinidés herbivores, et enrichis par des micro-organismes présents dans l'eau.

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A culture method for rearing mixed cyprinid species with different feeding habits has existed in China since the second century B.C. (Hu and Zhou 1989; Yang et al. 1992). This method utilizes allochthonous materials (e.g., land plants, waterweeds, snails, and/or bran) to increase fish production above that occurring naturally. The method has been improved empirically to obtain high fish production, but the energy flow through the pond ecosystem and the mechanism(s) by which such ponds can support high productivity remain unknown (Li 1987).

Typically, these ponds receive abundant submerged macrophytes (mainly *Vallisneria spiralis*) on which grass carp (*Ctenopharyngodon idella*) and blunt snout bream (*Megalobrama amblyocephala*) feed. Planktivorous silver (*Hypophthalmichthys molitrix*), bighead (*Aristichthys nobilis*), and crucian carp (*Carassius auratus*) are also reared in the ponds but do not receive a dietary supplement. According to Wu (1964a, 1964b), silver carp feed on phytoplankton and zooplankton, bighead

[1]Dedicated to the memory of Dr. Tsutomu Sasa (1920–1992).

[2]Present address: Lake Biwa Museum Project Office, 14-15 Uchidehama, Otsu, 520 Japan.

carp feed mainly on zooplankton, and crucian carp feed on algae, detritus, and/or crustaceans. However, the food selectivity of these species can change with food availability and the presence of other fish (e.g., Mizuno 1973). Little is known about how these fish species share or utilize the food in mixed culture ponds. The feeding habits of cyprinids have been investigated by microscopic observation of the gut contents (Wu 1964a, 1964b; Mizuno 1973; Kajak et al. 1977; Spataru 1977; Opuszyński 1979a; Bitterlich and Gnaiger 1984), but identification of the digested materials remaining in the alimentary tract is often difficult. In particular, zooplankton are often digested in the alimentary tract, so their importance as food for cyprinids has remained unclear (Bitterlich 1985).

Analysis of plant pigments in fish guts and faeces may help quantify the feeding habits of cultured cyprinids. All the cyprinid species lack a stomach, a structure that usually has gastric glands secreting HCl and pepsin. Iwata (1976, 1977) and Iwata et al. (1989) found that chlorophyll scarcely degraded to phaeopigments during passage through the intestines of the phytoplanktivorous crucian, silver, and bighead carp. On the other hand, pheophorbide and pheophytin were shown to be good indicators of herbivorous grazing by zooplankton and decomposition of phytoplankton or aquatic plants (Daley 1973; Daley and Brown 1973; Shuman and Lorenzen 1975; Carpenter and Bergquist 1985). Diatoms produce chlorophyllide due to high chlorophyllase activity (Jeffrey and Hallegraeff 1987). These data indicate that analysis of chlorophyll derivatives in fish faeces might identify the source of food for each fish. Moreover, part of the phytoplankton egested by these cyprinid species is alive and has photosynthetic activity (Iwata 1977; Bitterlich 1985; Miura and Wang 1985; Iwata et al. 1989), suggesting that the high photosynthetic rate in faeces may indicate herbivory on phytoplankton. The present study describes a novel approach using these pigment ratios and photosynthetic rates of gut material to quantify feeding habits of cyprinid species in a Chinese integrated fish culture pond.

Study Site

The carp culture pond (56 × 88 × 2.5 m) lies about 50 km east of Shanghai City and is connected by channels to Lake Dianshanhu (31°10′N, 120°90′E). Fish fry (483 kg of grass carp, 190 kg of blunt snout bream, 290 kg of silver carp, 860 kg of bighead carp, and 220 kg of crucian carp) were placed in the pond during December and January. Fish were harvested from the pond for commercial use on three occasions (June, July, and August) before our final survey, with a total of 1417 kg of silver and bighead carp and 383 kg of grass carp and blunt snout bream being removed. The pond was fertilized once a year with about 5 t of duck faeces in December before the fry were added. The plants added ($3.0\ t{\cdot}ha^{-1}{\cdot}d^{-1}$) were changed from terrestrial species (*Lolium perenne*) in April and May to aquatic species (mainly *V. spiralis*) from June to November.

Materials and Methods

Sampling and Environmental Conditions

The study was carried out in August 1991. Water temperature at a depth of 0.5 m at the center of the pond and the light intensity above the pond were measured at 10-min intervals with a thermistor thermometer (RMT; Rigosha and Co., Ltd.) and a quantum sensor (Koito IKS-25) equipped with a portable printer (YEW model 2543), respectively, during measurements of photosynthesis in phytoplankton and gut contents of fish to check the in situ conditions.

Water samples were collected from the surface to 2.4 m depth at the center of the pond with an acrylic tube sampler 5.0 cm in diameter. Six to 11 individuals of each cyprinid species were collected on 12, 21, 22, and 30 August from the pond before noon with a seine net. Some individuals were also collected on the evening of 29 August. The fish were dissected to obtain the contents of the fore and hind parts of the intestine soon after being caught. Each sample was divided into three parts. The first was fixed with glutaraldehyde (electron microscope grade, final concentration 2–2.5%) for microscopic enumeration of gut contents, the second was frozen at −20°C prior to measuring chlorophyll derivatives, and the third was suspended in pond water that had been filtered using Whatman GF/F filters for the measurement of photosynthesis.

Microscopic Observation of Gut Contents

Prior to microscopic enumeration, all sample bottles were shaken to suspend the gut contents evenly. The relative abundance of each taxon was determined by counting the number of squares occupied by each taxon in the eyepiece grid, using 30–50 haphazardly selected microscope fields at 100 or 200×.

Measurement of Chlorophyll Derivatives

Phytoplankton in water samples was collected quantitatively on 0.2-μm Nuclepore filters. The size fractionation of phytoplankton was made with 40-μm-mesh nylon cloth and 10- and 2-μm-pore Nuclepore filters using a handy vacuum pump (10–30 cm Hg vacuum). The filter and the sample of gut contents for each individual fish were extracted with 100% acetone to avoid formation of 10-methoxy and/or 10-methoxy lactone chlorophyll *a* (Otsuki and Takamura 1988). Chlorophyll was extracted at −20°C for 24 h without grinding. A Waters liquid chromatograph equipped with model 6000 A and M-45 pumps, an automated gradient controller (model 680), a U6K injector, and a Hitachi F-1050 fluorescence spectrophotometer were used. Wavelengths of 430 nm for excitation and 670 nm for emission were selected for the detection of chlorophyll derivatives. A two-step solvent program was used to separate dephytolated pigments such as chlorophyllide *a*, chlorophyll *c*, and pheophorbide *a*. After sample injection, solvent system A (90:5:5 methanol – ion-pairing solution (Mantoura and Llewellyn 1983) – distilled water) was pumped for 10 min, followed by solvent system B (80:20 methanol–ethanol) for 65 min at a flow rate of $1.0\ ml{\cdot}min^{-1}$. The ion-pairing solution consisted of tetrabutylammonium hydroxide (10 mL of a 0.5 M solution) and ammonium acetate (7.7 g) made up to 100 mL with distilled water. The solution was neutralized with acetic acid to pH 7.1. Solvent systems were switched with an automated gradient controller. The packed column was a Unisil NQ C18 (octadecychlorosilane bonded beads, 5 μm in diameter, 6.0 mm inside diameter × 150 mm in length; Gasukuro Kogyo Inc., Japan). Chlorophyll *a* and *b* standards (Sigma Chemical Co.) were prepared in 100% acetone and calibrated spectrophotometrically (Lichtenthaler 1987). The pheophytin *a* and *b* standards were prepared from chlorophyll *a* and *b* standards (Sigma Chemical Co.), respectively, by acidification with HCl and calibrated in 100% acetone spectrophotometrically (Lichtenthaler 1987). Chlorophyll *c* was extracted from *Chattonella antiqua* (NIES-1 of NIES Collection) and purified by

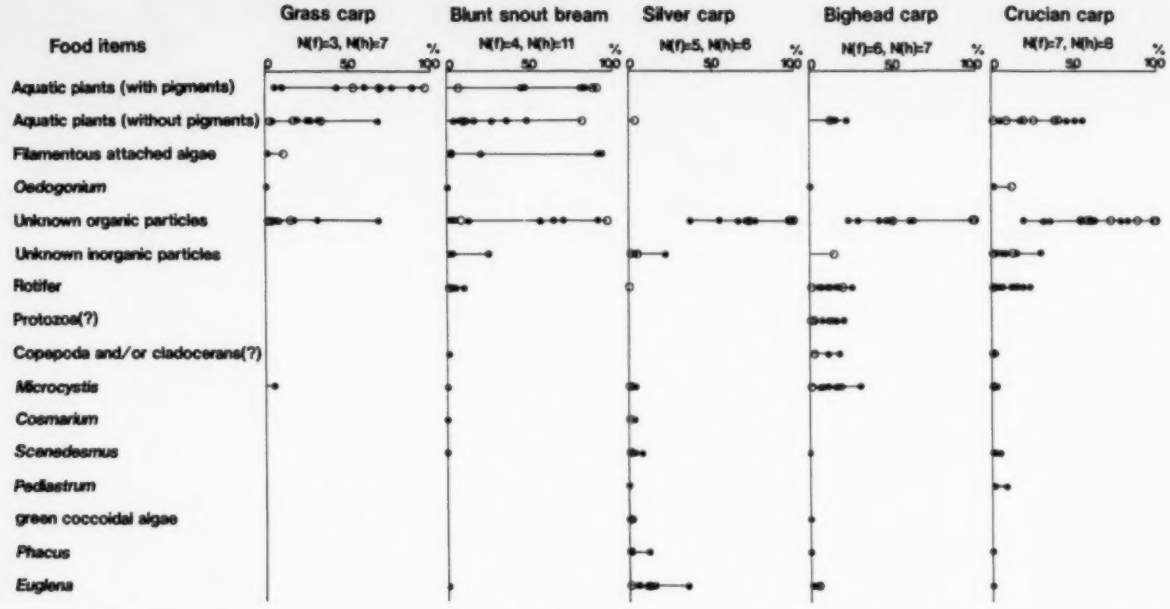

FIG. 1. Relative abundance (%) of food items in the gut contents of grass carp (*Ctenopharyngodon idella*), blunt snout bream (*Megalobrama amblycephala*), silver carp (*Hypophthalmichthys molitrix*), bighead carp (*Aristichthys nobilis*), and crucian carp (*Carassius auratus*). Only items representing 2% or more of the total are presented. Open circles indicate fore parts and closed circles indicate hind parts of intestines. Bars indicate ranges of the values. (Other items present in the guts of each fish species are as follows. Grass carp: *Spirogyra* and *Cocconeis*; blunt snout bream; *Spirogyra, Phormidium, Chamaesiphon, Cocconeis, Gomphonema, Cymbella, Navicula, Merismopedia, Nitzschia, Ankistrodesmus*, and *Dictyosphaerium*; silver carp: *Gomphosphaeria, Merismopedia, Cyclotella, Nitzschia, Ankistrodesmus, Chlamydomonas, Coelastphaerium, Crucigenia, Dictyosphaerium, Kirchneriella, Oocystis, Staurastrum, Trachelomonas*, blue-green dinoflagellata, and yellow algae (*Cryptomonas*?); bighead carp: *Ulothrix, Melosira, Crucigenia, Dictyosphaerium*, and blue-green dinoflagellata; crucian carp: *Ulothrix, Merismopedia, Ankistrodesmus, Crucigenia, Dictyosphaerium, Trachelomonas*, blue-green dinoflagellata, and yellow algae.)

TLC (Barrett and Jeffrey 1971). For identification purposes, chlorophyllide *a* was extracted from *Skeletonema costatum* (NIES-16 of NIES Collection). Chlorophyllides *a* and *b* were also prepared from chlorophyll *a* and *b* standards and the chlorophyllase enzyme obtained from *Citrus unshiu* fruits (Shimokawa 1982). Pheophorbide *a* was prepared by acidification of chlorophyllide *a*.

Measurement of Photosynthesis

Each sample of faeces, pond water, or pieces of aquatic plants was put into a 100-mL BOD bottle. The concentrations of chlorophyll *a* in the samples of gut contents were adjusted to become similar to those in the pond water. Photosynthesis was measured in situ just below the water surface for about 2 h in the afternoon at the center of the pond.

Just before the measurement, 0.2 mL of $NaH^{13}CO_3$ solution (99.0% atom; 5 $mg \cdot mL^{-1}$) was added to each bottle. After incubation, the samples were transferred immediately to a dark box and then collected on precombusted Whatman GF/F filters in a laboratory near the pond. Concentrations of organic carbon and the isotope ratios of ^{13}C and ^{12}C in the phytoplankton were determined by a quadrupole mass spectrometer equipped with a combustion furnace (Anelva TE-150) according to Otsuki et al. (1983). The photosynthetic rate (P_v, grams carbon per cubic metre per hour) was calculated according to Hama et al. (1983). The photosynthetic rates (P_{chl}, grams carbon per gram Chl *a* per hour) were obtained by dividing P_v by the concentrations of chlorophyll *a* (grams per cubic metre). The chlorophyll *a* concentrations of the samples, collected quantitatively on a Whatman GF/F filter, were measured by HPLC as described previously. Inorganic carbon (IC) was measured with a TOC analyzer (O.I. Corporation model 700).

Results

Observation of Gut Contents

Figure 1 shows the relative abundance (percent) of representative food items in the gut contents of each fish species. Most samples contained a high percentage of unidentified organic material except for several individual grass carp and blunt snout bream. The intestines of grass carp contained many fragments of aquatic plants, both with and without pigments. The guts of blunt snout bream were also full of plant fragments. However, the food composition of this species was more diverse and variable than that of grass carp. Filamentous algae (Xanthophyceae) occupied the hind part of the intestines of two individuals collected on 29 August, and unknown detritus, filamentous algae, and rotifers dominated four samples of the hind part of the intestine. Food selectivity was not related to fish size. We conclude that the selectivity for aquatic plants in blunt snout bream was not as strong as that in grass carp.

Many phytoplankton species were observed in the faeces of silver carp, although amorphous organic particles were

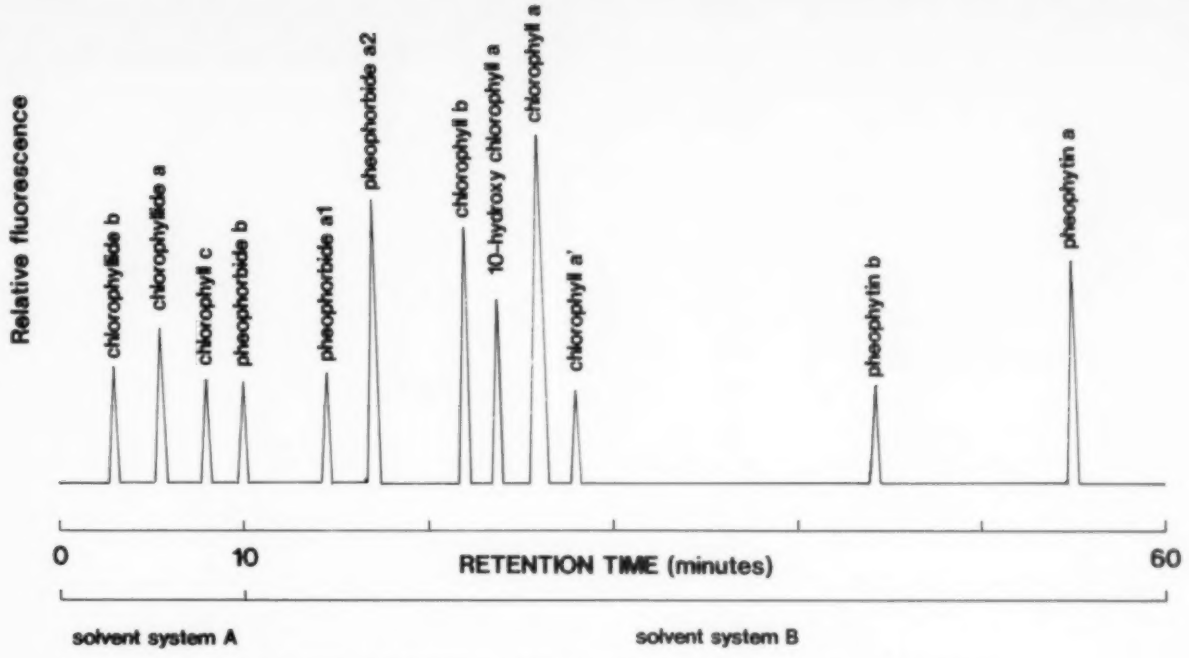

FIG. 2. Separation of pigments and pheopigments by HPLC from the standards in this study.

predominant. However, the amount of blue-green algae in the gut was less than that in the pond water (H.Q. Yang, unpublished data). Zooplankton and plant fragments were rarely observed. The fore part of the intestine of silver carp was mostly full of amorphous organic matter. Faeces of bighead carp contained a high percentage (16–57%) of zooplankton such as rotifers (*Anuroeopsis fissa, Schizocerca diversicornis,* and *Trichocerca* spp.), trophi of rotifers (*Asplanchna brightwelli* and *Brachionus* spp.), pieces of copepods and/or cladocerans, and organisms resembling protozoans. This species did not ingest the small phytoplankton as did silver carp, but took the large phytoplankton (*Microcystis* and *Euglena*) more frequently than silver carp. This is consistent with Iwata's observation (1976) that the distance between the gillrakers of silver carp is narrower than that of bighead carp. It is noteworthy that a considerable amount of plant fragments without pigments was observed in half of the bighead carp sampled.

The crucian carp consumed food similar to that of bighead carp. However, the intestine of this species contained more plant fragments and amorphous detritus and less zooplankton and phytoplankton than that of bighead carp. Material resembling protozoans, pieces of copepods and/or cladocerans, and *Microcystis* colonies were rare. Unidentified inorganic particles resembling sand were observed clearly, probably because this species often swallows sediments.

Measurements of Chlorophyll Derivatives

Analyses of our samples showed that the major form of pheophorbide *a* was not pheophorbide *a*1, as obtained in vitro after acidification of chlorophyllide *a*, but pheophorbide *a*2, which was later identified by its absorbance spectrum with a spectrophotometer (Fig. 2). Vernet and Lorenzen (1987) identified three major forms of pheophorbide (*a*1, *a*2, and *a*3) and reported that pheophorbide *a*2 was obtained in vitro by acidification of chlorophyllide *a* and that pheophorbide *a*3 was most abundant in *Calanus pacificus* fecal pellets. The pheophytin form observed in our samples was consistent with the peak obtained in vitro after acidification of chlorophyll *a*. Chlorophyllide *a* was only a minor component.

The Chl *b*/Chl *a* ratios of 10–40 μm phytoplankton (0.17 ± 0.04, mean ± SD, $n = 6$) were clearly higher than those of total (0.11 ± 0.03, $n = 6$), >40 μm (0.09 ± 0.09, $n = 4$), 2–10 μm (0.10 ± 0.03, $n = 6$), and <2 μm (0.08 ± 0.04, $n = 6$) sizes of phytoplankton because this size fraction is composed of *Euglena, Crucigenia Oocystis, Scenedesmus,* and *Westella*. However, the ratios of the aquatic plants (0.42 ± 0.07, $n = 6$) were significantly higher than those of the 10 – 40 μm phytoplankton. The pheophorbide *a*/Chl *a* (0.024 ± 0.006, $n = 6$) and pheophytin *a*/Chl *a* (0.004 ± 0.002, $n = 6$) ratios of the particulate matter in the pond water were very low. The aquatic plants contained little pheophorbide *a* (the pheophorbide *a*/Chl *a* ratio; 0.008 ± 0.006, $n = 6$), but some pheophytin *a* was found in three samples (Fig. 3c).

The three kinds of pigment ratios in the gut contents of grass carp were quite similar to those of the aquatic plants, indicating that this species feeds mainly on aquatic plants (Fig. 3). The ratios for blunt snout bream were not as uniform as those for grass carp. Most samples that had many plant fragments, such as those from grass carp, contained much chlorophyll *b* (0.35–0.51 Chl *b*/Chl *a* ratio) but few chlorophyll *a* derivatives. Another three samples with low Chl *b*/Chl *a* ratios (0.05–0.08) corresponded to those in which many filamentous algae were observed. A considerable amount of pheophorbide *a* was found in two samples (Fig. 3b).

The Chl *b*/Chl *a* ratios of the gut contents of silver carp (0.27 ± 0.06, $n = 6$) were intermediate between those of the 10 – 40 μm phytoplankton and those of the aquatic plants.

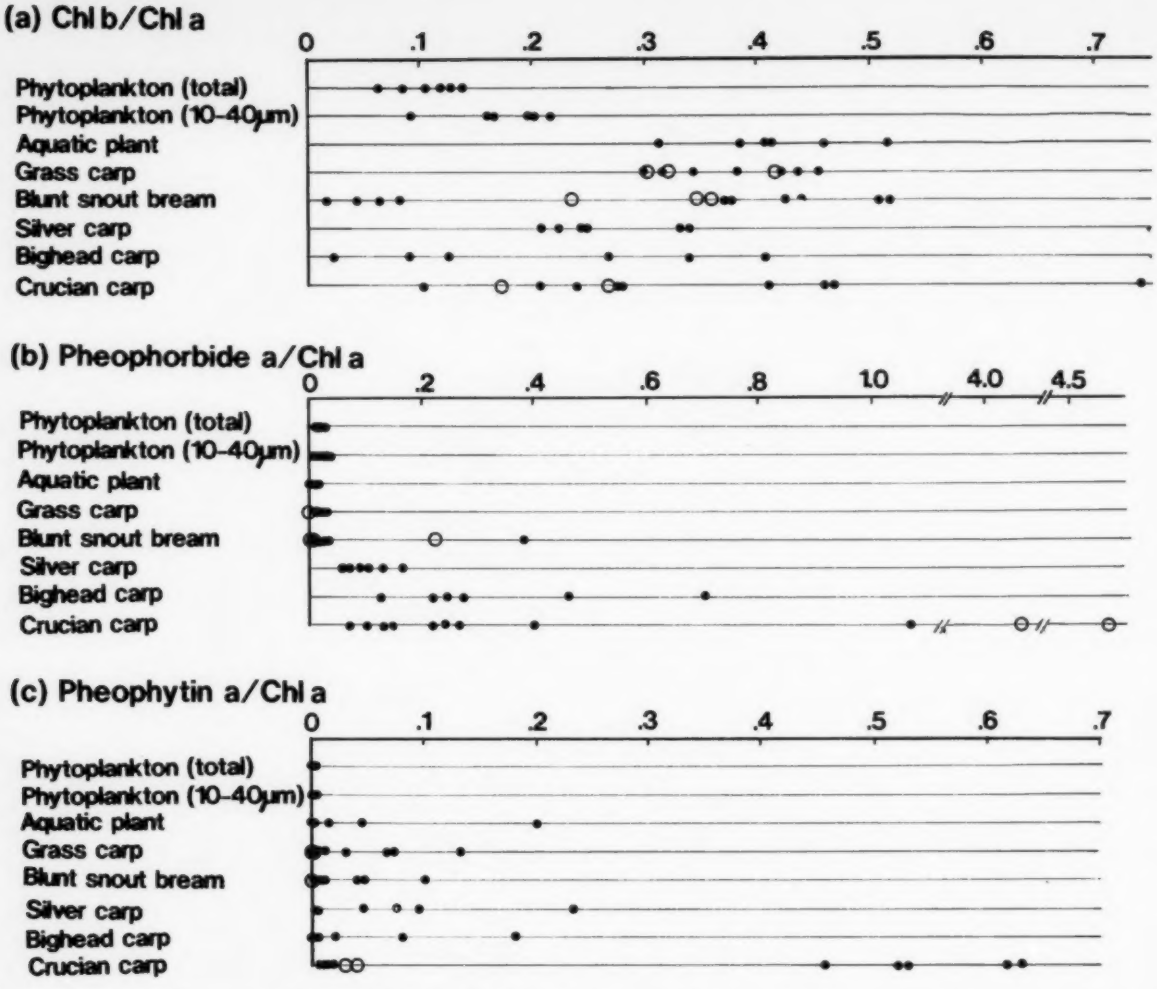

FIG. 3. Ratios (weight/weight) of chlorophyll *b* to chlorophyll *a* (including chlorophyll *a'* and 10-hydroxy chlorophyll *a*), pheophorbide *a* to chlorophyll *a*, and pheophytin *a* to chlorophyll *a* of particulate material in the pond water, aquatic plants, and guts contents of each cyprinid species. Open circles indicate fore parts and closed circles indicate hind parts of intestines.

TABLE 1. Photosynthetic rates (g C·g Chl a^{-1}·h^{-1}) of phytoplankton, aquatic plants, and faeces of each cyprinid species (N = number of samples).

Sample	n	Mean ± SE
Phytoplankton (total)	6	5.93 ± 0.43
Phytoplankton (10 – 40 μm)	5	6.33 ± 0.90
Aquatic plants	6	0.54 ± 0.09
Grass carp	10	0.09 ± 0.03
Blunt snout bream	18	0.24 ± 0.09
Silver carp	10	2.74 ± 0.31
Bighead carp	10	1.28 ± 0.17
Crucian carp	15	1.13 ± 0.20

Those of bighead and crucian carp ranged widely (Fig. 3a), but the samples with higher Chl *b*/Chl *a* ratios corresponded to those containing many plant fragments, although little pigment was observed.

The range and scattering of the pheophytin *a*/Chl *a* ratios were similar among aquatic plants and gut contents of grass carp, blunt snout bream, silver carp, and bighead carp; however, those of crucian carp varied more widely (Fig. 3c). The ranges of the pheophorbide *a*/Chl *a* ratios in samples of silver carp were narrow (0.06–0.17), while those of bighead carp ranged from 0.12 to 0.71, and those of crucian carp were the widest (0.08–4.57). Some higher ratios of pheophytin *a*/Chl *a* and pheophorbide *a*/Chl *a* of crucian carp may indicate that this species often swallows sediment. Crucian carp seemed to be the heaviest detritus feeder among five cyprinids in this pond.

Measurement of Photosynthesis

The photosynthetic rates of phytoplankton were about an order of magnitude higher than those of aquatic plants (*V. spiralis*) (Table 1). The faeces of grass-eating cyprinids scarcely photosynthesized. The faeces of silver carp had the highest photosynthetic rate of the five cyprinid species, although

the rates were still below half those of the phytoplankton. The photosynthetic rates of bighead and crucian carp faeces were one fifth the values for phytoplankton. It seems that the higher the photosynthetic rates of the faeces, the more phytoplankton the fish had taken.

Miura and Wang (1985) showed that the O_2 production rates per unit chlorophyll *a* in the faeces of silver and bighead carp were 74 and 200% as high as those for the equivalent-sized phytoplankton on which the carp grazed in East Lake, China. Presumably the photosynthetic rates of faeces of these cyprinid species depended largely on the quality and quantity of the dominant phytoplankton in the faeces.

Discussion

Pigment ratios (Chl *b*/Chl *a* and pheophorbide *a*/Chl *a*) and the photosynthetic rate of gut contents may help to explain the food utilization of cultured cyprinids. Pheophorbide *a* is known to be produced by herbivorous zooplankton (Daley 1973; Shuman and Lorenzen 1975; Carpenter and Bergquist 1985; Conover et al. 1986) and may be a promising index of grazing intensity by herbivorous zooplankton (Carpenter and Bergquist 1985). In the present study, concentrations of pheophorbide *a* were high in the gut contents of some bighead and crucian carp. The guts of these fish also contained many rotifers, indicating that these carp species may feed on herbivorous zooplankton. The occurrence of pheophorbide *a* in the faeces of silver carp showed that this species also utilized herbivorous zooplankton, although the zooplankton itself was rarely observed in the faeces. Lack of zooplankton remains may reflect rapid digestion of zooplankton, as observed by Bitterlich and Gnaiger (1984).

In our samples, pheophytin *a* was probably derived from both herbivorous grazing and the decomposition of aquatic plants. Pheophytin *a* is also produced during the digestion of algae by herbivores (Daley 1973; Carpenter and Bergquist 1985; Vernet and Lorenzen 1987). The ratio of pheophytin *a* to pheophorbide *a* formed by herbivorous grazing seemed to vary according to the type of grazer (Carpenter and Bergquist 1985), gut passage time, and time of exposure to degradation in the environment (Vernet and Lorenzen 1987). However, pheophytin cannot be used as an indicator of herbivory because it is also produced by exposing algae to darkness for a long period and during cell lysis by bacteria and viruses (Daley 1973; Daley and Brown 1973). Pheophytin *a* was also found in some samples of aquatic plants offered to grass-eating cyprinids as food, indicating that these plants may be partially decomposed.

The major foods of silver carp have been reported to be phytoplankton, zooplankton, and detritus, with selection depending on environmental conditions (see Kajak et al. 1977). Our results are consistent with this. Of the five cyprinid species studied, silver carp were shown to utilize phytoplankton most frequently and to prefer Chlorococcales and Euglenophyceae to blue-green algae. This agrees with Kajak et al. (1977), who found that silver carp fed mainly on phytoplankton even if algae were rare and that this species showed selectivity for particular species of phytoplankton, preferring diatoms, Chlorococcales, and Euglenophyceae, but avoiding blue-green algae, in particular filamentous blue-green algae. Heavy blooms of blue-green algae have been shown to interrupt feeding and to delay growth and increase rejection of food (Savina 1965, cited in Kajak et al. 1977; Omarov and Lazareva 1974, cited in Kajak et al. 1977).

Iwata (1976) showed that the distance between the gillrakers of silver carp is 10–15 μm for 40–50 mm body length and 18–22 μm for 230 mm body length, indicating that this species is not able to collect the smaller phytoplankton. Primary production for this pond in summer is not as high (0.5–2.0 g $C \cdot m^{-2} \cdot d^{-1}$) as reported for other hypertrophic lakes (Takamura et al. 1992). Moreover, most phytoplankton are <10 μm because of high grazing pressure on larger sized phytoplankton (Takamura et al. 1992; N. Takamura, unpublished data). Furthermore, it is reported that the majority of planktivorous fish can digest diatoms but not Chlorococcales, Euglenophyceae, or blue-green algae (Prowse 1964; Savina 1965, cited in Kajak et al. 1977; Spataru 1977; Bitterlich 1985). Indeed, we observed photosynthesizing Chlorococcales and Euglenophyceae phytoplankton in the faeces of silver carp, indicating that the algae of this group remained undigested during passage through the intestine. It is suggested that the amounts of phytoplankton utilized by silver carp were insufficient for the growth of silver carp in this pond.

Silver carp in this pond utilized herbivorous zooplankton. It has been reported that zooplankton were eaten when preferred algae were rare, especially if blue-green algae dominated the phytoplankton (Omanov and Lazareva 1974, cited in Kajak et al. 1977; Bergun 1975, cited in Kajak et al. 1977; Kajak et al. 1977). Thus, the food conditions for silver carp may not be optimal in this pond ecosystem.

Both the higher ratios of Chl *b*/Chl *a* and pheophytin *a*/Chl *a* and the lower photosynthetic rates (based on the amount of chlorophyll *a*) in the faeces of silver carp, as compared with the same values in phytoplankton, suggest the possibility of feeding on detritus derived from plant fragments. The significance of detritus in the food of silver carp has been discussed by many authors (see Opuszyński 1979a) because amorphous organic matter has often been found in the alimentary tract, similar to the results of the present study. Although Lin et al. (1981) denied any caloric gain from detritus by silver carp, the consensus is that detritus is generally nutritious for silver carp depending on its quality (Opuszyński 1979a, 1979b). Dead or decomposing algae are assimilated better than live algae (Vovk 1974, cited in Kajak et al. 1977; Barthelmes and Jahnichen 1978, cited in Bitterlich 1985).

In the study, the biomass of both large-sized phytoplankton and zooplankton was very small (Takamura et al. 1982; H.Q. Yang, unpublished data) mainly due to high grazing pressure by planktivorous cyprinids, which in turn results in a chronic lack of food. However, this pond receives a lot of plant debris every day in the form of faeces of grass-eating cyprinids. The high Chl *b*/Chl *a* ratios found in the gut contents of planktivorous cyprinids indicated that they feed on the faeces of grass-eating cyprinids. However, these planktivorous fish species did not themselves digest plant fragments containing much cellulose. Iwata et al. (1992) found that the amino acid contents of the faeces of grass carp increased gradually during a time-course laboratory experiment on cell lysis of the faeces egested by grass carp in aerated pond water. They found active uptake of ^{3}H-labeled thymidine and ^{15}N-labeled ammonium by bacteria attached to the egested plant debris. It is suggested that these planktivorous fish species mainly utilized the attached nutritive microorganism community. Furthermore, the bacterial production in this pond was very high (at least 7–10 mg $C \cdot m^{-3} \cdot h^{-1}$ in August) (N. Takamura, unpublished data), suggesting a rich bacterial energy source. Therefore, bacteria in this pond not only mineralized plant fragments, but also were directly utilized

by planktivorous fish, as they grew on plant fragments. A large amount of the plant debris originating in the faeces of grass-eating cyprinids was considered to pass through the alimentary tract of planktivorous carp after being nourished by microorganisms in the pond water. The plant debris essentially provided the substrate by which the bacteria's energy could be transferred to a higher trophic level. The bacteria themselves are too small to be consumed directly by planktivorous fish. Presumably this food chain can transfer energy more efficiently than a more typical "grazing" food chain. This is one mechanism in mixed cyprinid culture systems, documented in the ancient Chinese saying: "Grass carp cultivate planktivorous carp."

Pigment analysis clarified the origin of unidentified detritus in the guts of several cyprinids which had heretofore been overlooked by microscopic observation. Our techniques have provided an effective method to quantify the feeding habits of cyprinids species.

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Element Concentrations in Chironomids and Their Abundance in the Littoral Zone of Acidified Lakes in Northwestern Ontario[1]

Vincent L. St. Louis[2]

Department of Zoology, University of Toronto, Toronto, Ont., Canada M5S 1A1

St. Louis, V.L. 1993. Element concentrations in chironomids and their abundance in the littoral zone of acidified lakes in northwestern Ontario. Can. J. Fish. Aquat. Sci. 50: 953–963.

I studied species diversity of the chironomid community, abundance of emerging adults, and accumulation of metals and elements in chironomids in the shallow littoral zone of experimentally acidified and unmanipulated reference lakes at the Experimental Lakes Area in northwestern Ontario. Orthocladiinae were significantly more abundant in acid lakes than in reference lakes, while the Chironominae were less numerous. The lower abundance of Chironominae resulted from fewer chironomids in the Tribe Tanytarsini. Chironomini were more common in acid lakes than in the reference lakes. Biomass of emerging chironomids either increased significantly following acidification or was not different from that of reference lakes. Concentrations of Al, Ca, Mn, and Zn were on average higher in chironomids from a number of the acid lakes than in chironomids from reference lakes. Calcium concentrations in chironomids from the most acid lake were significantly lower, however, suggesting that at low pH levels, Ca may be difficult to sequester. Increased accumulation of certain metals in chironomids, coupled with increased biomass of emerging chironomids as well as low levels of Ca, makes chironomids a potentially abundant but poor-quality food source for organisms that rely on them as prey.

J'ai étudié la diversité des espèces de chironomidés, l'abondance des adultes émergents et l'accumulation des métaux et des éléments dans la zone littorale peu profonde de lacs acidifiés expérimentalement et de lacs de référence non manipulés de la Région des Lacs Expérimentaux dans le nord-ouest de l'Ontario. Les Orthocladiinae étaient substantiellement plus abondants dans les lacs acidifiés que dans les lacs de référence, alors que les Chironominae étaient moins nombreux. La plus faible abondance des Chironominae était attribuable à une sous-représentation des chironomidés de la tribu des Tanytarsini. Les Chironomini étaient plus fréquents dans les lacs acidifiés que dans les lacs de référence. La biomasse des chironomidés émergents augmentait de manière substantielle après l'acidification ou bien ne différait pas de celle des lacs de référence. Les concentrations de Al, Ca, Mn et Zn étaient, en moyenne, plus élevées chez les chironomidés présents dans un certain nombre de lacs acidifiés que chez les chironomidés provenant des lacs de référence. Toutefois, la concentration de Ca chez les chironomidés du lac le plus acide était substantiellement plus faible, ce qui indiquerait qu'à faible pH, la séquestration du Ca peut être plus difficile. Une accumulation accrue de certains métaux chez les chironomidés, combinée à une augmentation de la biomasse des adultes émergents et à une faible concentration de Ca, fait des chironomidés une source d'aliment potentiellement abondante, mais de qualité médiocre pour leurs prédateurs.

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Anthropogenic acidification of aquatic ecosystems may affect the invertebrate community as a food source for higher trophic levels by (1) eliminating important acid-sensitive prey, (2) changing abundance of the remaining acidophilic species (e.g., through shifts in predator–prey communities), (3) increasing bioaccumulation of potentially toxic metals that are often leached from sediments, soils, or bedrock or transported with acid industrial emissions, and (4) decreasing levels of certain essential nutrients, such as calcium, in prey (e.g., Eriksson et al. 1980; Campbell and Stokes 1985; Økland and Økland 1986; Schindler 1988a).

Chironomids (Diptera: Chironomidae) emerging from lakes are a major food source for aerial foraging insectivorous birds, such as tree swallows (*Tachycineta bicolor*), nesting near lakes (Quinney and Ankney 1985, St. Louis et al. 1990, Blancher and McNicol 1992). For example, St. Louis et al. 1990 showed that tree swallows first foraged on the abundant chironomids that emerged at their nest-site lake before searching for food patches elsewhere. Blancher and McNicol (1988) found that in central Ontario, wetland pH was positively related to clutch size, clutch volume, and nestling growth in tree swallows. They suggested that reduced availability of acid-sensitive prey items, especially those rich in Ca, near acid wetlands may have contributed to decreased reproductive success (Blancher and McNicol 1992). However, Blancher and McNicol (1992) found that the proportion of chironomids in the diet of swallows near acid wetlands was actually greater than in the diet of swallows near circumneutral wetlands where acid-sensitive prey species were more common. St. Louis et al. (1993) showed a significant negative relationship between nest-site lake pH and levels of liver metallothionein, a metal-binding protein, in nestling swallows. Metallothionein concentrations were correlated with hepatic concentrations of Cu and Zn, suggesting increased dietary metal intake by nestlings near acidified habitats.

[1]This paper is one of a series done in honour of the 25th anniversary of the Experimental Lakes Area.

[2]Current address: Department of Microbiology, University of Manitoba, Winnipeg, Man., Canada R3T 2N2.

TABLE 1. Size, maximum depth (Z) (from Cleugh and Hauser 1971), and time-weighted mean (1986–89) chemical characteristics (ELA Station, unpublished data following techniques outlined in Stainton et al. 1977) of the study lakes.

	Size (ha)	Z (m)	NO_3^--N ($\mu g \cdot L^{-1}$)	NO_2-N ($\mu g \cdot L^{-1}$)	TDN ($\mu g \cdot L^{-1}$)	TDP ($\mu g \cdot L^{-1}$)	DIC ($\mu mol \cdot L^{-1}$)	DOC ($\mu mol \cdot L^{-1}$)	SO_4^{2-} ($mg \cdot L^{-1}$)	Cl^- ($mg \cdot L^{-1}$)	Na^+ ($mg \cdot L^{-1}$)	K^+ ($mg \cdot L^{-1}$)	Mg^{2+} ($mg \cdot L^{-1}$)	Ca^{2+} ($mg \cdot L^{-1}$)	Fe^{2+} ($mg \cdot L^{-1}$)	Mn^{2+} ($mg \cdot L^{-1}$)	Cond. ($\mu S \cdot cm^{-1}$)	Alk. ($\mu Eq \cdot L^{-1}$)	pH
Acid lakes																			
L302S	10.9	10.6	16	228	451	3	61	279	12.07	0.73	0.94	0.43	0.73	2.71	0.14	0.28	43	13	4.93
L302N	12.8	13.8	246	91	590	4	60	465	4.91	3.18	0.89	0.47	0.75	2.43	0.07	0.25	34	14	5.58
L223	30.2	11.2	30	45	250	2	37	287	11.18	0.31	1.11	0.37	0.68	2.99	0.04	0.08	37	8	5.72
L225	5.6	1.8	40	50	535	6	80	833	4.99	0.98	0.88	0.32	0.64	1.51	0.09	0.03	25	11	5.17
Reference lakes																			
L114	12.1	5.0	29	129	507	3	58	605	4.23	0.28	0.72	0.57	0.62	1.60	0.07	0.02	23	42	6.35
L224	25.4	16.7	5	24	178	2	106	240	3.41	0.37	0.82	0.38	0.51	2.03	0.05	0.02	22	88	6.86
L303	9.9	2.5	12	47	369	3	78	549	4.03	0.27	0.73	0.64	0.66	1.90	0.05	0.03	23	72	6.58
L304	3.6	6.7	6	25	435	5	103	757	2.73	0.40	1.04	0.64	0.79	1.86	0.11	0.01	25	104	6.81
All reference lakes (average)			13	56	372	3	86	538	3.60	0.33	0.83	0.56	0.64	1.85	0.07	0.02	23	77	6.65

This study was initiated as part of a larger project examining the effects of lake acidification on the reproductive success of birds (St. Louis 1992). Here I examine in detail the abundance of emerging adults and concentrations of elements in chironomids in acidified lakes. I also assess the species diversity of the chironomid community to investigate possible changes in chironomid abundance or element concentrations resulting from acidification.

Methods

Study Site

This study was conducted from 1986 to 1989 at the Experimental Lakes Area (ELA) in northwestern Ontario (93°30′–94°00′W and 49°30′–49°45′N) (Schindler 1988b, 1990). Morphological and chemical characteristics of the study lakes are presented in Table 1. Briefly, the chironomid community was examined in four reference lakes (L114, L224, L303, and L304; time-weighted mean 1986–89 epilimnia pH levels ranged between 6.4 and 6.9 among these lakes), one lake naturally acidified with high organic acids (L225; mean pH 5.2), and three experimentally acidified lakes (L302S, L302N, and L223). The epilimnion of L223 was acidified with H_2SO_4 from pH 6.6 in 1976 to pH 5.1 during 1981–83 (Schindler et al. 1985); the pH was then allowed to recover to values of 5.5–5.9 during 1984–89. L302 is a double-basin lake divided into halves by a seacurtain. Beginning in 1982, the south basin (L302S) was acidified by about pH $0.25 \cdot yr^{-1}$ with H_2SO_4 to pH 4.8–4.5 in 1988–89. The north basin (L302N) received additions of HNO_3 until 1986 (pH 6.1) and then HCl to pH 5.8–5.1 during 1987–89 (Rudd et al. 1990).

Chironomid Collection

Adult chironomids were collected using transparent funnel traps (basal area 0.1 m^2) suspended 1 m below the lake surface, as described by Davies (1980). Three traps were placed equidistant from each other in the 2-m depth zone of each lake because the density of emerging chironomids is generally very high along this depth contour (Davies 1980).

Because I was interested in the element concentrations in chironomids as well as their abundance and species composition, care was taken throughout the sampling operation to avoid element contamination of the chironomids. All equipment used in the handling of chironomids was initially acid washed by soaking in 15% HNO_3 for a minimum of 48 h, followed by at least five rinses with deionized water.

Traps were set by attaching an inverted acid-washed glass jar with a trapped airspace to the funnel for a period of approximately 48 h during each week from June to July 1986 and from May to July 1987–89. In the laboratory, contents of each jar were sieved onto a plastic 300-μm mesh, rinsed into a plastic petri dish with deionized water, counted (see below), and transferred to an acid-washed polypropylene cryotube using glass forceps. Labware was acid washed each day and rinsed with deionized water between samples. Samples were frozen at −20°C at the ELA and later stored at −80°C until analysed for element contents.

Chironomid Species Diversity

To determine if lake acidification caused changes in chironomid diversity, chironomids collected in 1986–87 and 1989

were identified to the species level by B. Bilyj, Freshwater Institute, Winnipeg, Man. In 1986–87, all chironomids were transferred with deionized water from their cryotube to an acid-washed plastic petri dish, identified, immediately returned to the cryotube, and refrozen as described above for future element analyses. In 1989, I set emergence traps weekly for a second 24-h period to obtain samples for species identification and dry weight biomass estimates (see below). Chironomids collected during this time were preserved with 70% ethanol in plastic cryotubes.

I calculated the frequencies of species emerging from each of the acid and reference lakes among all years. Diversity of chironomid taxa in each of the lakes was calculated with Simpson's index of diversity (Simpson 1949).

For each Subfamily and Tribe of chironomids, I tested for differences in frequencies between each acid lake and the mean for reference lakes using *G*-tests of independence (Sokal and Rohlf 1981). However, because of the large number of *G*-tests performed, *P*-values were adjusted for each Subfamily or Tribe to an overall $\alpha = 0.05$ level using the sequential Bonferroni method (Rice 1989). I calculated means for reference lakes and not acid lakes for this analysis and analyses described below because while there was little difference among the reference lakes in natural pH, the acid lakes varied widely in both acidification treatment and pH. This technique allowed for a direct comparison of reference lakes with the different acid treatments.

Abundance of Chironomids

Chironomids were divided into three size categories: 0–2.5 mm (small), >2.5–5.0 mm (medium), and >5.0 mm (large). Biomass (B) of chironomids (milligrams dry weight) emerging at each trap site per square metre every hour was calculated using the formula

$$B = 10\left(\frac{(N_S \times W_S) + (N_M \times W_M) + (N_L \times W_L)}{t}\right)$$

where N_S, N_M, and N_L are the numbers of small, medium, and large chironomids collected in the trap during each trapping period, W_S, W_M, and W_L are the average dry weights of chironomids in the three size categories, t is the exact number of hours each trap was set, and the multiplier 10 converts the trap area from 0.1 to 1 m^2.

Dry weights were estimated using the chironomids collected in 1989 preserved in ethanol (however, fats were possibly leached from the tissues by the ethanol). For each lake, replicates (number depended on number of chironomids collected) of 1–10 chironomids per size class were placed in preweighed foil pouches and dried at 65°C for 48 h in a drying oven. Mean dry weight of chironomids for each size class and lake was then calculated. Later, I pooled the dry weights in each size category among the study lakes because there were too few samples for some lakes. Average dry weight (milligrams ± SE) of chironomids in each size category was $W_S = 0.027 \pm 0.003$, $W_M = 0.096 \pm 0.013$, and $W_L = 0.270 \pm 0.025$.

The biomass of chironomids emerging at each of the three trap sites at a lake was averaged weekly ($\bar{B}$). Average biomass of chironomids emerging weekly among the four reference lakes was calculated. Average biomass of chironomids emerging each year was compared between each acid lake and the reference lakes using two-tailed *t*-tests, with *P*-values derived from a randomization test (10 000 iterations) (Edgington 1987). However, because of the large number of *t*-tests performed, *P*-values were again adjusted for each lake to an overall $\alpha = 0.05$ level using the sequential Bonferroni method.

Element Composition of Chironomids

For each lake, I pooled samples collected in weeks (after ice-out) 5–7 and 8–11 in 1986, 2–4, 5–7, and 8–11 in 1987, 1–4, 5–7, and 8–11 in 1988, and 1–3, 4–6, and 7–11 in 1989 to obtain a sufficient dry weight sample for element analyses. These three periods each year corresponded to the three stages of the tree swallow reproductive cycle (egg laying, incubation, and nestling feeding). Ice-out was during the week of 26–27 April in 1986–87, 1 May in 1988, and 7 May in 1989. Chironomids were dried to constant weight at 65°C for 48 h in a drying oven. Dried chironomids were transferred with a teflon-coated spatula to waxed weighing paper, weighed on a CAHN model 4100 electrobalance accurate to 0.001 mg, and placed in an Erlenmeyer flask covered with a watchglass. I used on average 2.51 ± 0.17 mg (± SE) dried chironomids for all analyses. Twenty chironomid samples were digested coincidently, along with two samples of National Bureau of Standards oyster tissue standard reference material (SRM) and two acid blanks. On average, 14.05 ± 0.75 mg SRM was analysed.

An initial subset of chironomids collected during 1986–88 was digested in 50-mL glass Erlenmeyer flasks. However, I had poor recovery of S with the SRM and could not detect Al. Therefore, the remainder of the 1986–88 samples and all the 1989 samples were digested in 50-mL teflon Erlenmeyer flasks. To each flask I added 4 mL of a 4:1 trace-grade HNO_3 – $HClO_4$ solution. Samples were digested overnight (approximately 15 h) at room temperature. The following day, when digested in glass flasks, samples were taken to dryness on a teflon-coated hotplate over a period of 6–8 h, diluted with exactly 4.5 mL of a 6% trace-grade HNO_3 – deionized water solution, and then stored in acid-washed plastic vials until analysis. When digested in teflon flasks, samples were removed from the hotplate when approximately 0.3 mL of solution remained (dryness could not be obtained). Samples were then diluted to exactly 5 mL in an acid-washed volumetric flask with deionized water (giving a final solution of approximately 6% acid) and transferred to acid-washed plastic vials. SRM and blank samples were treated similarly. All samples were refrigerated until analysed (up to 2 mo).

Samples were analysed for Al, Ca, Cd, Cu, Fe, Mg, Mn, P, Pb, S, and Zn using inductively coupled plasma argon emission spectrophotometry (ICPES). Samples digested in glass flasks were analysed with an ARL 34000 spectrophotometer at the Institute of Environmental Studies, University of Toronto, Toronto, Ont., while those digested in teflon flasks were analysed with a Thermo Jarrell Ash AtomScan 25 spectrophotometer at the Ministry of the Environment, Toronto, Ont. Concentrations of elements in chironomids were corrected for background contamination by subtracting values from blanks. Concentrations below detection limits were replaced with detection-limit values calculated by multiplying the machine detection limit for the given element (0.0001–0.002 $\mu g \cdot g^{-1}$) with the dilution factor of the sample (e.g., 5 mL) and then dividing by the weight of the sample. The resulting sample value was the highest possible concentration for that sample.

Prior to detailed analyses of elemental composition of chironomid tissues, I corrected for differences in percent recovery of elements from the SRM digested in glass and in recovery of elements from the SRM digested in teflon flasks. Recovery of

TABLE 2. Number of species of chironomids (relative frequencies in parentheses) in each Subfamily and Tribe identified from acid and combined reference lakes (also see Appendix). *Relative frequency significantly different ($P \leq 0.05$) from the frequency in reference lakes; **relative frequency significantly different when the P-value was adjusted to an overall $\alpha = 0.05$ level using the sequential Bonferroni method.

	Acid lakes				
	Acidified L302S (mean pH 4.93)	Acidified L302N (mean pH 5.58)	Acidified L223 (mean pH 5.72)	L225 (natural mean pH 5.17)	Reference lakes[a] (mean pH 6.35–6.86)
Subfamily Tanypodinae	10 (9.56)*	9 (8.20)	4 (5.14)	4 (2.66)**	7.0 (6.62)
Tribe Procladiini	4 (4.23)	2 (3.35)	2 (2.23)	2 (0.97)*	3.5 (2.76)
Tribe Pentaneurini	6 (5.33)	7 (4.85)	2 (2.91)	2 (1.69)*	3.5 (3.86)
Subfamily Orthocladiinae	12 (16.55)**	11 (39.99)**	9 (11.17)**	5 (21.27)**	6.5 (4.26)
Subfamily Chironominae	41 (73.89)**	42 (51.81)**	35 (83.69)**	27 (76.07)**	26.25 (89.12)
Tribe Pseudochironomini	12 (0.58)	1 (0.81)	1 (3.60)**	0 (0.00)	0.5 (0.19)
Tribe Chironomini	22 (40.30)**	23 (20.89)	18 (44.27)**	12 (51.03)**	11.5 (24.37)
Tribe Tanytarsini	18 (33.01)**	18 (30.11)**	16 (35.82)**	15 (25.04)**	14.25 (64.56)
Total number of species	63	62	48	36	39.75

[a]Values are averages of L114, L224, L303, and L304 (see Appendix).

Ca, Fe, Mg, Mn, and P was 14.6, 1.8, 2.0, 8.0, and 11.2% higher, respectively, in teflon flasks than in glass flasks, while recovery of Cu and Zn was, respectively, 2.6 and 3.1% lower. I also tested for differences in elemental composition of chironomids used for identification (chironomids collected in 1986–87) and those not used (collected in 1988–89) by comparing mean tissue concentrations for chironomids collected from reference lakes using two-tailed t-tests, with P-values derived from randomization tests (10 000 iterations). I assumed that little variation occurred among years in element concentrations in chironomids in the reference lakes.

For each acid lake and the combined reference lakes, the mean concentration was calculated for each element for each period and year. A mean concentration ($\bar{C}$; micrograms per gram) was then calculated for each element among all years and period for each acid lake and for the pooled reference lakes. For each element, I tested for differences in $\bar{C}$ between each acid lake and the pooled reference lakes using t-tests, with P-values derived from randomization tests (10 000 iterations). Because certain metals may leach from lake sediments following acidification, I predicted that metal concentrations would be greater in chironomids from acid lakes than in chironomids from reference lakes. I therefore used one-tailed tests to examine metal concentrations. However, because Ca may actually initially increase in water following acidification due to increased weathering of lake sediments (see Schindler 1988a) before possibly becoming difficult for biota to sequester, I used two-tailed tests when examining this element. I also used two-tailed tests to compare concentrations of P and S in chironomid tissues because I had no a priori prediction of direction. Because of the large number of t-tests performed, P-values were adjusted for each element to an overall $\alpha = 0.05$ level using the sequential Bonferroni method. Furthermore, to determine if there was an overall direction in accumulation or loss of elements from chironomids from acid lakes compared with reference lakes, I combined the P-values from acid lakes using Fisher's (1970) technique (also see Sokal and Rohlf 1981).

Results

Chironomid Species Diversity

One hundred and twenty species of chironomids were identified emerging from the 2-m depth zone among all lakes during the sampling period (Appendix). The total number of species of chironomids found in each experimentally acidified lake was greater than the average number in the reference lakes, but less in naturally acid L225 (Table 2). Chironomid community diversity ranged between 0.80 for the naturally acid L225 and reference L303 and 0.95 for the experimentally acidified L302N and reference L224 (Appendix). The Chironominae dominated the chironomid community among all the study lakes. However, the Chironominae were significantly less abundant in the acid lakes than in the reference lakes, while the Orthocladiinae were significantly more abundant (Table 2). Tanypodinae were significantly more abundant in acidified L302S than in the reference lakes. Frequency of the Tanypodinae was significantly lower in L225 than in the reference lakes, resulting from chironomids in the Tribes Procladiini and Penteurini being less abundant there (Table 2). Within the Chironominae, the Chironomini were more abundant (except for the Chironomini in L302N) in the acid lakes, while the Tanytarsini were less abundant than in the reference lakes (Table 2). No one species was more common in the acid lakes than in the reference lakes, and within each lake, only a few species made up more than 10% of the population (Appendix).

Abundance of Chironomids

Emergence from the reference lakes appeared to increase in 1988–89 over the two previous years (Fig. 1); however, sampling missed the early peaks of emergence following ice-out in 1986–87. A significant increase in chironomid abundance in L302S occurred in 1988–89 as the pH of the lake was decreased to 4.8 and 4.5, respectively, in comparison with abundance in the reference lakes (Fig. 1). Abundance of chironomids emerging from L302N remained relatively constant among sampling years and was significantly higher than that from the reference lakes in 1986 only. Emergence from L225 was more variable among years. Abundance and patterns of emergence from L223 as the pH of the lake recovered from pH 5.6 to 5.9 seem to be comparable every second year (Fig. 1).

Element Composition of Chironomids

Recovery of the different elements from the SRM ranged between 81 and 99% when chironomids were digested in glass flasks and between 86 and 100% when digested in teflon flasks.

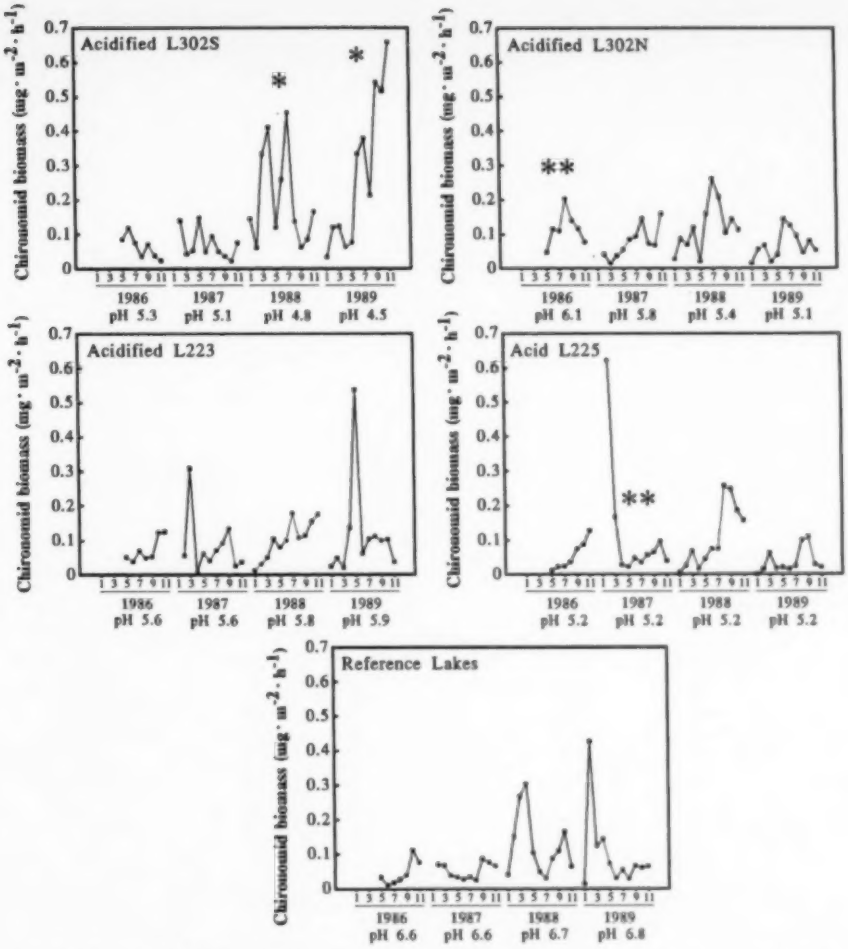

FIG. 1. Biomass ($\bar{B}$) of chironomids emerging from the acid lakes and combined reference lakes during 1986–89. The abscissa depicts weeks past ice-out in any given year. *Years when biomass was significantly different ($P < 0.05$) from that in the reference lakes; **significantly different when P-values were adjusted to an overall $\alpha = 0.05$ level with the sequential Bonferroni method.

I found significantly lower concentrations of Mg ($t = 17.55$, $P = 0.002$), P ($t = 14.40$, $P = 0.003$), and S ($t = 3.31$, $P = 0.020$) in tissues of chironomids used for identification, possibly due to leaching of these elements in deionized water during identification. Therefore, chironomids collected in 1986–87 were omitted from analyses for these elements. I found no significant difference ($P \geq 0.05$) in concentrations of any of the other elements.

Cadmium and Pb were omitted from analyses because concentrations in chironomid tissues were always below or near-detection limits. Concentrations of Al and Cu were below their detection limit in 14.1 and 15.3% of the samples, respectively. These missing values were replaced with detection limit concentrations (see Methods) for statistical analyses.

Concentrations of Al, Mn, and Zn were on average significantly higher in chironomids emerging from the 2-m depth zone of the acid lakes than in those emerging from the similar zone of the reference lakes using Fisher's (1970) method of combining probabilities (Table 3). However, few of the concentrations in chironomids from specific acid lakes were significantly different from mean concentrations in chironomids collected from the reference lakes (Table 3), indicating that variation in concentrations among years and periods was high. Manganese concentrations were significantly higher in chironomids collected from experimentally acidified lakes, but lower at naturally acid L225, than in chironomids from the reference lakes. Because concentrations of Mg in chironomids were significantly higher at L225, but not different at experimentally acidified lakes from concentrations in chironomids at the reference lakes, it appears that lake acidification does not result in consistent accumulation of this metal in chironomids. Calcium concentrations were significantly higher in chironomids from experimentally acidified L302N and L223, but significantly lower in L302S, the most acid lake (Table 3).

Discussion

Chironomid Species Diversity

In a review of the literature on the effects of pH on benthic fauna, Økland and Økland (1986) generalized that although

TABLE 3. Mean (± SE) concentrations ($\bar{C}$; $\mu g \cdot g^{-1}$) of metals (upper section of table) and elements (lower section) in chironomids. The number of samples (periods and years) contributing to the mean concentrations (also see Methods and Results) is given in parentheses. The mean concentrations of metals at the acid lakes were compared with the mean concentrations of metals at the reference lakes using one-tailed *t*-tests whereas the mean concentrations of elements were compared using two-tailed *t*-tests (see Methods). *Significantly different from reference lakes at $P < 0.05$; **significantly different when the *P*-values were adjusted to an overall $\alpha = 0.05$ level using the sequential Bonferroni method; †significantly different from the acid lakes using Fisher's (1970) method of combining probabilities (see Methods).

	Acid lakes				
	Acidified L302S (mean pH 4.93)	Acidified L302N (mean pH 5.58)	Acidified L223 (mean pH 5.72)	L225 (natural mean pH 5.17)	Reference lakes (mean pH 6.35–6.86)
Al	63.31 ± 15.07* (9)	57.94 ± 14.16 (8)	84.61 ± 36.27 (11)	62.65 ± 13.42* (10)	34.01 ± 6.52† (10)
Cu	18.07 ± 2.77 (11)	17.51 ± 2.72 (11)	19.19 ± 3.13 (11)	18.22 ± 3.59 (11)	17.22 ± 2.59 (11)
Fe	719.87 ± 91.15 (11)	721.55 ± 81.13 (11)	769.06 ± 76.97 (11)	440.66 ± 52.08 (11)	633.87 ± 42.26 (11)
Mg	1069.32 ± 45.47 (6)	1078.67 ± 62.23 (6)	1046.88 ± 40.08 (6)	1289.45 ± 57.76* (6)	1146.13 ± 21.18 (6)
Mn	45.49 ± 7.85** (11)	49.19 ± 7.34** (11)	34.81 ± 6.21** (11)	13.31 ± 1.50 (11)	17.98 ± 1.32† (11)
Zn	143.92 ± 12.01 (11)	169.86 ± 9.63** (11)	141.66 ± 11.58 (11)	117.63 ± 8.84 (11)	129.65 ± 7.03† (11)
Ca	933.48 ± 59.48* (11)	1540.28 ± 158.03* (11)	1548.30 ± 166.07* (11)	1285.09 ± 118.25 (11)	1138.36 ± 74.56 (11)
P	9059.34 ± 327.08 (6)	9489.26 ± 520.15 (6)	9770.19 ± 489.04 (6)	10850.89 ± 949.86 (6)	9194.64 ± 148.03 (6)
S	5286.34 ± 112.46 (6)	5387.95 ± 218.65 (6)	5341.89 ± 139.84 (6)	5571.33 ± 197.46 (6)	5208.03 ± 80.08 (6)

decreasing pH was usually associated with decreasing numbers of species, in the case of some invertebrates tolerant to low pH, abundance may increase. The chironomid communities in acid lakes in the Adirondacks (Uutala 1980), Maine (Hunter et al. 1985), Sweden (Henrikson et al. 1982), and the Netherlands (Leuven et al. 1987) all exhibited reduced number of species and increased abundance of a few species.

The relative abundance of chironomids of the Subfamily Chironominae in the littoral zone of the acid lakes at the ELA was lower than at the reference lakes, resulting primarily from a lower abundance of chironomids of the Tribe Tanytarsini. In an earlier study of the chironomid community at all depths in experimentally acidified L223 at the ELA, Davies (1985) found that Tanytarsini accounted for more than half of the total emergence as the pH of the lake was lowered from 6.7 to 5.2 (even though the number of species in this Tribe decreased from 29 to 6) whereas members of the Chironomini, Tanypodinae, and Orthocladiinae showed only small responses to acidification. Bilyj and Davies (1989) later described seven new species of *Cladotanytarsus* from L223, six of which I also found in samples from both the acid and reference lakes. The species described by Bilyj and Davies (1989) displayed various degrees of tolerance to acidification. *Cladotanytarsus muricatus*, the species missing from my samples, was the most acid sensitive (disappearing at pH 6.5) whereas *C. aeiparthenus*, the most abundant species in the experimentally acidified lakes, was the most acidophilic (greatest abundances found at pH levels around 5.1).

No individual species was consistently abundant in all acid lakes examined at the ELA. Other studies in which chironomid communities in acid lakes were analysed also showed inconsistencies in the dominant Subfamily, Tribe, and to a greater extent species. In North America, for example, Uutala (1980) found that species of Tanytarsini (*Tanytarsini* spp.), Orthocladiinae (*Heterotrissocladius changi*), and Chironomini (*Polypedilum* sp. and *Chironomus anthracinus*) were common in an upper profundal site in an acid lake (pH 4.5–5.3) in the Adirondacks, but species of Procladiini (*Procladius* spp.) dominated the chironomid community in a circumneutral lake. Similarly, Bendell-Young and Harvey (1988) found that the Chironomini and Tanytarsini dominated the chironomid community in acid lakes in south-central Ontario, but circumneutral lakes contained a greater diversity of taxa. To the contrary, the Tanytarsini as well as *Chironomus salinarius* decreased and *C. anthracinus* increased in relative abundance in lakes with low pH in north-central Ontario (Dermott et al. 1986). Walker et al. (1985), however, found that *Procladius* dominated the chironomid community in acid lakes in Atlantic Canada. As in my study, species of Chironomini (*Chironomus* and *Cladopelma*) and Orthocladiinae (*Monopsectrocladius*) were also more abundant in the acid upland lakes than the lakes of higher pH where species of Tanytarsini (*Paratanytarsus*) and Chironomini (*Pagastiella*) were more abundant.

Because of these discrepancies in chironomid community composition among acid sites in North America, including locally acidified lakes at the ELA, it is tempting to speculate that the chironomid community in acid lakes is determined by the community prior to acidification. Certainly, the number of species found in the reference lakes overall shows sufficient diversity to give rise to different new communities if the pH were to be changed. The high number of chironomid species in the shallows of the experimentally acidified lakes at the ELA is possibly the result of a transition between the abundance of acid-sensitive and acidophilic species or vise versa in L223, which is presently being allowed to recover in pH. L225 is historically acid and therefore most likely maintains a stable community structure of acidophilic species.

Abundance of Chironomids

Biomass of chironomids emerging from the 2-m depth zone either dramatically increased following acidification (L302S) or did not change significantly. Although shifts in biomass of emerging adult chironomids may be related to the differences in community composition described above, other factors such as production of phytoplankton (chironomid resources: Davies 1980; Welch et al. 1988) and abundance of certain species of fish (chironomid predators: Økland and Økland 1986) are known to affect emerging chironomid abundance. Because phytoplankton production at the ELA did not show any clear response to lake acidification (e.g., Shearer et al. 1987), the significant increase in chironomid abundance at L302S in 1988–

89 was probably influenced by the sudden decline in fish abundance in those years (Mills and Chalanchuk 1991). Similar dramatic increases in chironomid abundance did not occur in L302N and L223. In the early stages of experimental acidification of L223, Schindler et al. (1985) found that chironomid emergence increased as the pH was lowered from 6.8 to 5.6, roughly in proportion to phytoplankton production. As the pH of the lake was further lowered to 5.1, however, emergence declined to levels observed in the reference lakes. Abundance of fish in L223 never reached levels as low as those found in L302S (Mills et al. 1987; Mills and Chalanchuk 1991). During the years of this study, the abundance of fish either changed little or increased in L302N and L223. However, emergence abundance in L223 appeared to follow a 2-yr pattern of cycling, possibly the result of species in the community with 2-yr life cycles. Unfortunately, I was not able to examine shifts in species abundance because I did not identify chironomids in 1988, although most temperate species of chironomids are either uni- or bivoltine (Pinder 1986).

Variation in chironomid abundance also exists among acid sites outside the ELA. For example, while Henrikson et al. (1982) found a decrease in the total abundance of chironomids in two acid lakes in Sweden, Harvey and McArdle (1986) found that chironomid density was not pH related in lakes in south-central Ontario. Bendell-Young and Harvey (1988) later found chironomid abundance to be nearly five times greater in two of three acid lakes than in two circumneutral lakes.

Element Composition of Chironomids

Increased concentrations of certain metals have been found in acidified waters, and these increases have been attributed to direct industrial contamination by precipitation or to the leaching of metals from soils, bedrock, and sediments (LaZerte 1986). At the ELA, atmospheric deposition of pollutants is minimal (Linsey et al. 1987), and therefore the only source of greatly increased metals in acidified lake water is mobilized from lake sediments.

Concentrations of Al, Mn, and Zn in adult chironomids emerging from the shallow littoral zone were on average higher in most of the acid lakes in this study than in the reference lakes, but concentrations of Cu, Fe, S, and P in chironomids appeared to be unaffected by lake acidification. Manganese showed the most pronounced accumulation of all metals among the experimentally acidified lakes, most likely reflecting high levels of Mn oxides that were stored in sediments of ELA lakes (Schindler et al. 1980) and high concentrations of Mn in the epilimnetic water of the acidified lakes in this study (Table 1). Unfortunately, we do not have similar water column concentrations of Al and Zn. Schindler et al. (1980), however, found that Al, Mn, and Zn were released from sediments in enclosure experiments when the pH level of the overlying water was decreased to either 6 or 5.

Bendell-Young and Harvey (1988) also found that chironomid adults from an acid lake contained greater concentrations of Zn than those from circumneutral lakes. Manganese and Al were lower in adult chironomids from the acid lakes, however, and similar to results of this study, Fe and Cu concentrations did not differ among the lakes. Krantzberg and Stokes (1988) found that Al, Cd, Cu, Fe, Mn, Ni, and Pb body burdens in adult chironomids were higher at pH levels 4.4–5.5 than at pH levels greater than 5.8 in lakes in south-central Ontario. However, the highest body burdens of Cd, Al, Cu, and Ni were observed in organisms from metal-contaminated sites at pH 5.1.

Contrary to results from the experimentally acidified lakes, concentrations of Fe and Mn were lower and Mg significantly higher in chironomids from naturally acid L225 than in those from the reference lakes. Organic acids are responsible for the acidity of L225. Possibly these results are a function of the rich organic sediments found in L225 compared with the mineral sediments of the 2-m depth zone in the experimentally acidified lakes. Some elements are easily bound to organics (Campbell and Stokes (1985), possibly making them less biologically available. Water concentrations of Mn in L225, for example, were similar to those in the epilimnia of reference lakes. It is also possible that the chironomid species abundant in L225 (e.g., *Psectrocladius simulans* and *Pagastelia ostansa*), or this particular population of these species, are better adapted for regulation of these elements under acid conditions than individuals that dominate the experimentally acidified lakes. Krantzberg and Stokes (1989) found that larvae from metal-contaminated lakes were able to regulate their accumulation of Cu, Ni, and Mn.

Calcium concentrations in chironomids from experimentally acidified L302N and L223 were significantly higher than in chironomids from the neutral pH reference lakes, most likely reflecting the high levels of this element in the water of the acidified lakes (Table 1). It should be noted, however, that Krantzberg and Stokes (1989) found that chironomids in metal-contaminated sediments were more efficient at retaining or sequestering Ca than chironomids from noncontaminated areas. Calcium burdens were highest in chironomids at pH 4.4, possibly reflecting an adaptive response because Ca can protect against H^+ toxicity by interfering with the uptake of some cations (Krantzberg and Stokes 1988). Calcium concentrations in chironomids from the most acid lake (L302S), however, were significantly lower than in those from the reference lakes, suggesting that Ca may be difficult to sequester regardless of its availability (Table 1) or amelioration potential (also see Wiener and Stokes 1990). Reduced Ca metabolism is well documented for a number of other invertebrates in acid lakes (e.g., see Økland and Økland 1986).

Potential Consequences to the Reproductive Success of Birds Foraging on Chironomids

Lake acidification could affect the chironomid commuity as a food source for tree swallows by altering species composition of the community, abundance, nutrient quality, and/or accumulation of potentially toxic metals. Shifts I observed in the community composition to acidophilic species following lake acidification should have no direct effect on food abundance for swallows. In fact, these shifts most likely prevent depletion of a food source for tree swallows breeding near lakes following acidification (St. Louis et al. 1990; Blancher and McNicol 1992). Furthermore, inshore chironomid abundance did not appear to change, and may even increase, in later stages of lake acidification.

Increased accumulation of metals in and/or loss of nutrients from chironomids, however, may be a problem for consumers in higher trophic levels. The elevated concentrations of Zn, for example, amplified by the increased biomass of chironomids emerging from some acid lakes, may be partially responsible for the increased concentration of Zn in hepatic and renal tissues of tree swallow nestlings near the acid lakes in this study (St.

Louis et al. 1993). Certainly, as shown here, chironomids provide a route for metals such as Al, Mn, and Zn to leave acidified aquatic systems and enter the terrestrial ecosystem. While an increase in certain metals may lead to toxicity in birds (e.g., Scheuhammer 1991), the decrease in Ca concentration in chironomid tissue at very low pH levels would only confound the difficulty swallows already have finding Ca supplements near acid lakes which are needed by the birds for egg production and bone ossification (St. Louis and Breebaart 1991; Blancher and McNicol 1992) and probably act synergistically to increase the toxicity of metals (Scheuhammer 1991). Therefore, lake acidification appears to have an effect on chironomids in a way that may in turn reduce their quality as a food source for tree swallows and, by definition, for other organisms that rely on them as prey.

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Appendix

TABLE A1. Relative frequency of chironomid species in each acid and reference lake.

	Acid lakes				Reference lakes			
	L302S	L302N	L223	L225	L114	L224	L303	L304
Subfamily Tanypodinae	9.56	8.20	5.14	2.66	11.16	8.29	5.16	1.88
Tribe Procladiini	4.23	3.35	2.23	0.97	5.21	1.66	2.95	1.23
Procladius (Holotanypus) denticulatus	1.08	0.35		0.24	3.35	1.18	0.74	0.23
Procladius (Holotanypus) freemani								0.06
Procladius (Holotanypus) ruris	0.08				0.74			0.06
Procladius (Holotanypus) sublettei	0.08		0.17			0.24	1.47	0.59
Procladius (Psilotanypus) bellus	2.99	3.00	2.06	0.73	1.12	0.24	0.74	0.29
Tribe Pentaneurini	5.33	4.85	2.91	1.69	5.95	6.63	2.21	0.65
Ablabesmyia (s.s.) aspera	1.41	0.23		1.45	1.12	0.71	2.21	0.53
Ablabesmyia (s.s.) mallochi	1.25	1.50	2.74	0.24	0.37	2.13		0.06
Ablabesmyia (s.s.) monilis	0.17	0.69			1.86			
Ablabesmyia (s.s.) parajanta	1.75	0.58				0.24		
Ablabesmyia (s.s.) philosphagnos	0.50							
Ablabesmyia (s.s.) tarella	0.25	1.61						0.06
Conchapelopia currani						0.24		
Djalmabatista pulcher		0.12	0.17			0.24		
Guttipelopia guttipennis					2.60			
Larsia canadensis		0.12				3.07		
Subfamily Orthocladiinae	16.55	39.99	11.17	21.27	4.83	7.83	1.48	2.88
Corynoneura fittkaui	0.50		0.69		0.37		0.74	
Corynoneura lacustris	0.33	1.27	0.34			0.95		
Corynoneura scutellata			0.52			0.71		0.06
Corynoneura sp. 1	1.00	3.80	0.69		0.37			
Heterotanytarsus nudalus	0.50			0.24		0.24		
Heterotrissocladius changi		0.46	1.89			0.95		
Nanocladius (s.s.) incomptus		7.26				0.24		
Nanocladius (s.s.) rectinervis		0.58				0.24	0.74	0.12
Orthocladius (s.s.) annectens		0.12	0.52					
Parakiefferiella bathophilia				0.24				
Parakiefferiella cf. *coronata*	0.17	10.25	0.34			1.66		
Parakiefferiella sp. 1		0.12						
Psectrocladius (Mesopsectrocladius) sp. 1	0.17				1.49	0.24		
Psectrocladius (Monopsectrocladius) calcaratus	1.83			0.12	0.37			1.82
Psectrocladius (Monopsectrocladius) sp. 1				0.36	1.12			
Psectrocladius (Monopsectrocladius) sp. 2	0.08				0.74	0.47		
Psectrocladius (s.s.) oligosetus	8.31	1.61						0.06
Psectrocladius (s.s.) simulans	3.41	1.27	0.69	20.31	0.37	0.24		0.76
Psectrocladius (s.s.) sp. 1	0.08							0.06
Thienemanniella sp. 1		13.25	5.49			1.89		
Zalutschia sp. 1	0.17							
Subfamily Chironominae	73.89	51.81	83.69	76.07	84.01	83.88	93.36	95.24
Tribe Pseudochironomini								
Pseudochironomus atricaudus	0.58	0.81	3.60			0.71		0.06
Tribe Chironomini	40.30	20.89	44.27	51.03	38.66	28.15	7.37	23.31
Chironomus (s.s.) anthracinus	0.50							
Chironomus (s.s.) decorus	1.08							

TABLE A1. *(Continued)*

	Acid lakes				Reference lakes			
	L302S	L302N	L223	L225	L114	L224	L303	L304
Chironomus (s.s.) cf. *lacunaris*								
Chironomus (s.s.) plumosus								1.00
Chironomus (s.s.) tuxis	0.50			0.12				
Cladopelma amachareus	0.33			3.02	1.49	0.71		0.47
Cladopelma viridula				1.69				
Cryptochironomous conus	0.17		0.69	0.12		0.71		
Cryptochironomous obreptans		0.12		0.12				
Cryptochironomous scimitarsus	0.08	0.23	0.34					0.12
Cryptochironomus sorex	0.58	0.58	0.34		0.37			0.06
Demeijerea sp. 1		0.35						
Dicrotendipes milleri			0.17					
Dicrotendipes modestus	29.09	1.38	3.26	4.84	1.49	0.24	2.21	1.64
Dicrotendipes tritomus			1.20					
Endochironomous nigricans	0.08	0.23	0.17					
Einfeldia dissidens					0.37			
Einfeldia longipes	0.08							0.35
Glyptotendipes (Phytotendipes) lobiferus			2.40					
Glyptotendipes (Phytotendipes) paripes	0.08	0.12	0.17				1.47	1.93
Glyptotendipes (Phytotendipes) sp. 1			0.17					
Glyptotendipes (Phytotendipes) sp. 2		0.12						
Lauterborniella agrayloides					11.52	8.51		
Microtendipes pedellus pedellus	0.91				0.74		0.74	
Microtendipes pedellus stygius				1.21		3.07	0.74	4.51
Nilothauma babiyi	0.25	0.12	0.52			0.47		
Nilothauma mirabilis	0.75		0.69	0.73		1.18		0.47
Pagastiella ostansa	1.91	1.61		37.49		4.73		1.93
Parachironomus potamogetei				0.48				
Paracladopelma galaptera	0.25	0.35	0.52			0.24		
Paracladopelma undine		0.58				0.24		
Paralauterborniella nigrohalterale	0.08	3.57						
Paratendipes albimanus	1.50	0.12						
Paratendipes subequalis								0.06
Polypedilum (Pentapedilum) sordens		0.12	0.52			0.24		
Polypedilum (Pentapedilum) tritum	0.58	0.23		0.24				6.32
Polypedilum (Polypedium) nubeculosum					13.76		1.47	0.29
Polypedilum (Tripodura) albinodus		1.04						
Polypedilum (Tripodura) gomphus	0.75	2.88		0.97		5.91		
Polypedilum (Tripodura) scalaenum		4.49	25.73			0.24		
Polypedilum (Tripodura) simulans		1.61	1.20		1.86	0.71	0.74	4.10
Polypedilum (Tripodura) sp. 1	0.08	0.46						
Stenochironomus hilaris			0.52					
Stictochironomus sp. 1	0.67	0.46	5.66			0.24		
Tribelos jucundum		0.12			7.06	0.71		0.06
Tribe Tanytarsini	33.01	30.11	35.82	25.04	45.35	55.02	85.99	71.87
Cladotanytarsus aeiparthenus	8.23	5.88	2.06	0.12			3.68	
Cladotanytarsus daviesi		0.12	6.86			6.86		
Cladotanytarsus elaensis				0.36		0.95		
Cladotanytarsus fusiformis	0.50	1.73		0.36	3.72	3.07		7.79
Cladotanytarsus pinnaticornis	0.42	0.35	1.37					
Cladotanytarsus tribelus			0.17					37.41
Cladotanytarsus sp. 1				3.63	15.24	5.67	20.59	10.83
Cladotanytarsus sp. 2					1.12			5.21
Constempellina brevicosta							0.74	
Constempellina rodesta	0.08	1.27						
Micropsectra nigripila						0.24		
Micropsectra sp. 1		0.23						
Paratanytarsus cf. *bituberculatus*	0.08	2.77	1.72					
Paratanytarsus laccophilus	0.08							0.35
Paratanytarsus sp. 1	0.33							
Paratanytarsus sp. 2	0.17		0.17					
Stempellina cf. *bausei*	0.33	5.99	0.17	0.61		3.07		
Stempellinella leptocelloides	0.33	0.46	4.63	0.12		3.31		
Stempellinella sp. 1		1.15				2.84	0.74	0.18

TABLE A1. *(Concluded)*

	Acid lakes				Reference lakes			
	L302S	L302N	L223	L225	L114	L224	L303	L304
Tanytarsus cf. *curticornis*	8.90	1.27	4.46	0.24		1.42		
Tanytarsus debilis	1.16		0.17	5.20	1.12	1.66	3.68	3.63
Tanytarsus nr. *heusdensis*						0.71		
Tanytarsus lobiger	1.08		0.52	3.14		3.07		0.53
Tanytarsus medius		0.12				1.89	2.21	
Tanytarsus mendax	0.17			0.73		0.24	6.62	0.41
Tanytarsus nemorosus	5.57			0.24	1.12	1.18		0.82
Tanytarsus neoflavellus	0.33	0.12						
Tanytarsus niger								1.00
Tanytarsus norvegicus								0.23
Tanytarsus cf. *quadridentatus*	0.17	4.49	12.18	0.48	1.12	9.22	2.94	1.17
Tanytarsus recurvatus		0.12		0.12		0.95	6.62	
Tanytarsus cf. *signatus*		0.23	0.17			0.71		
Tanytarsus sp. 1	5.07	0.69	0.52	9.31	4.09	8.04	37.50	1.46
Tanytarsus sp. 2			0.34	0.36	2.60			0.53
Tanytarsus sp. 3					0.74			
Tanytarsus sp. 4			0.34		0.37			0.35
Tanytarsini gen. indet.		3.23					0.74	
Zavreliella sp. 1					14.13			
Total number of specimens	1203	868	583	827	269	423	136	1708
Species diversity (Simpson's (1949) index)	0.88	0.95	0.90	0.80	0.91	0.95	0.80	0.83

Comparison of Some Life History Characteristics of Pacific Herring (*Clupea pallasi*) from the Canadian Pacific Ocean and Beaufort Sea

R.W. Tanasichuk

Department of Fisheries and Oceans, Pacific Biological Station, Nanaimo, B.C., Canada V9R 5K6

A.H. Kristofferson

Department of Fisheries and Oceans, Freshwater Institute, Winnipeg, Man., Canada R3T 2N6

and D.V. Gillman

Department of Fisheries and Oceans, 867 Lakeshore Road, Burlington, Ont., Canada L7R 4A6

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We compared several growth and reproductive characteristics of Pacific herring (*Clupea pallasi*) from the Canadian Pacific Ocean and Beaufort Sea using data for 2310 fish gillnetted in the northern Strait of Georgia or near Tuktoyaktuk, N.W.T., over the 1985 spawning season. Weight-at-age was similar but Beaufort Sea fish were longer-at-age. Total weight-at-length was significantly greater for Strait of Georgia herring because their size-specific ripe ovary weights were 2.1 times greater. We attributed differences in growth and surplus energy allocation to adult instantaneous mortality rates being 1.8 times higher for Strait of Georgia herring. Size-specific ripe egg weight was 30% smaller in Beaufort Sea herring presumably because warmer sea temperatures over the larval period resulted in a higher mortality rate. Weight-specific fecundity was 1.5 times greater in Strait of Georgia fish. Ovarian growth rates near spawning were lower in Beaufort Sea herring because they mature in colder water.

Nous avons comparé plusieurs caractéristiques de la croissance et de la reproduction du hareng du Pacifique (*Clupea pallasi*) de l'océan Pacifique (partie canadienne) et de la mer de Beaufort à l'aide de données provenant de 2 310 poissons capturés au filet maillant dans la partie nord du détroit de Georgie ou près de Tuktoyaktuk, T.N.-O., au cours de la saison de frai de 1985. Le poids selon l'âge était semblable, mais la longueur selon l'âge des poissons de la mer de Beaufort était plus grande. Le poids total selon la longueur était statistiquement plus élevé dans le cas du hareng du détroit de Georgie parce que le poids des ovaires matures spécifiques de la taille était 2,1 fois plus élevé. Nous avons attribué les différences de croissance et d'attribution de l'énergie excédentaire au taux de mortalité instantané des adultes qui est 1,8 fois plus grand dans le cas du hareng du détroit de Georgie. Le poids des ovaires matures spécifique de la taille était plus faible de 30 % chez les poissons de la mer de Beaufort en raison, probablement, des températures de la mer plus élevées au cours de la période larvaire qui a entraîné une mortalité plus grande. La fertilité selon l'âge était 1,5 fois plus élevée chez les poissons du détroit de Georgie. Le taux de croissance ovarienne à l'approche du frai était plus faible chez le hareng de la mer de Beaufort parce que la maturation se fait dans des eaux plus froides.

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The biomass of Pacific herring (*Clupea pallasi*) inhabiting the British Columbian coast is about 150 000 tonnes (Haist and Schweigert 1990). Herring spawn during March–April at temperatures and salinities ranging from 7 to 9°C and 27 to 28.7 ppt, respectively (Alderdice and Hourston 1985). Most of these fish consist of stocks spawning along the southern British Columbian coast, including the northern Strait of Georgia. They rear and feed off the lower west coast of Vancouver Island, at approximately 48°N latitude, in waters averaging 8–9°C and 32 ppt. These herring feed between April and October, with euphausiids (*Thysanoessa spinifera*) dominating as prey (D.M. Ware and R.W. Tanasichuk, unpublished results). Herring off the lower west coast of Vancouver Island are important prey for the approximately 200 000 tonnes of Pacific hake (*Merluccius productus*) and 75 000 tonnes of spiny dogfish (*Squalus acanthias*) that feed there over summer (Tanasichuk et al. 1991).

Pacific herring inhabiting the Beaufort Sea experience very different living conditions. Herring normally spawn in June–July, at spring break-up, with spawning occurring at depths of the thermocline (1–5 m). Sea temperatures and salinities range from 5 to 12°C and 6 and 17 ppt, respectively (Shields 1985). Lawrence et al. (1984) showed that herring disperse along the Beaufort Sea coast, located at about 69°N, to feed after spawning. Water depths, temperatures, and salinities ranged between 0.7 and 9 m, 2.5 and 14.4°C, and 0.04 and 29.4 ppt, respectively, over the open-water period (June–September). These authors reported that herring in the Beaufort Sea have a diverse diet consisting of plankton (mainly copepods), benthic infaunal (Pelecypoda, Acarina, Nematoda, Chironomidae), and epi-

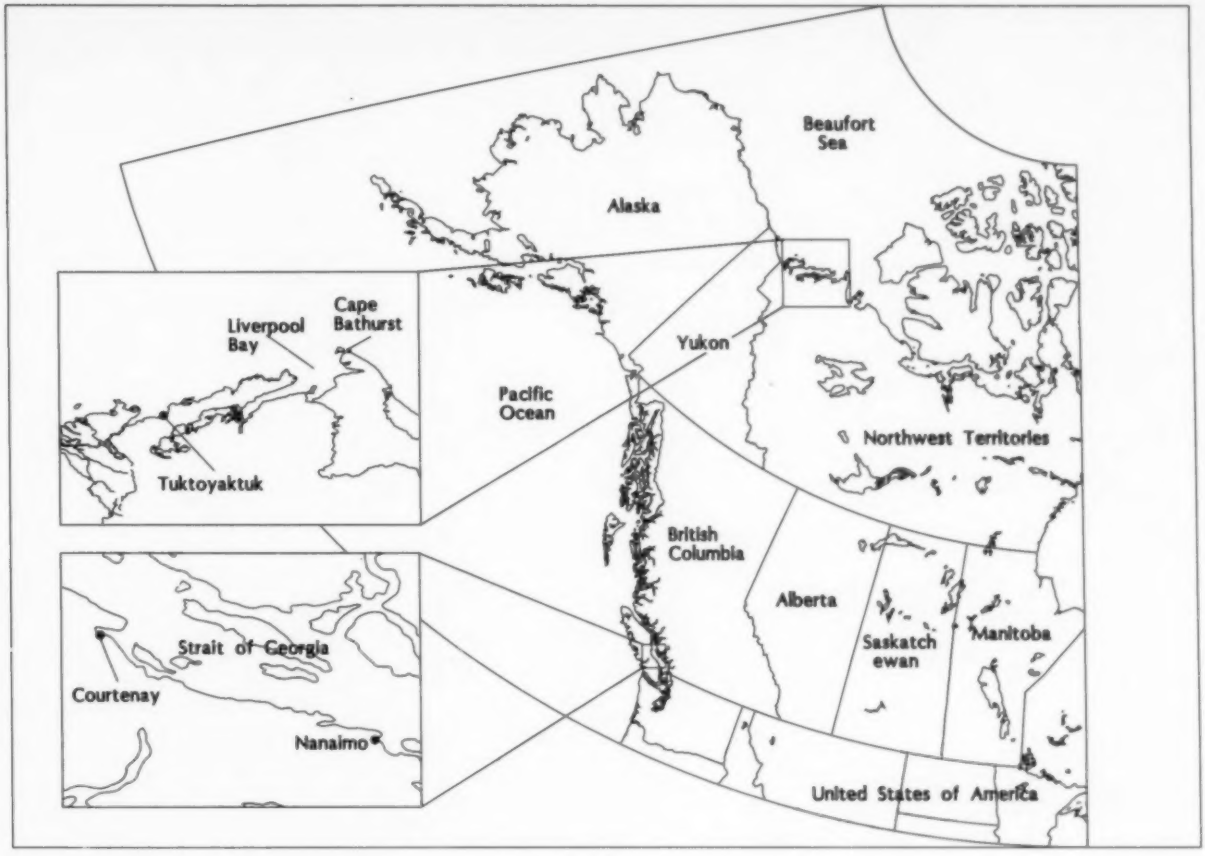

FIG. 1. Sampling locations

benthic organisms. They found that the only potential fish predator is inconnu (*Stenodus leucichthys*). Their distribution maps suggested that herring and inconnu distributions overlap in the summer (August) and fall (September). Beluga whales (*Delphinapterus leucas*) (Sergeant and Hoek 1974; Seaman et al. 1982; Lowry et al. 1986) and pinnipeds such as the spotted seal (*Phoca largha*) (Lowry 1985) also feed on herring. However, there is no information on how important herring are as prey and what consumption rates are. Finally, no one has estimated the biomass of herring living in the Beaufort Sea.

Information on the influence of latitude on the life history characteristics of Pacific herring is limited. Hay (1985) showed that spawning time varied and suggested that all Pacific herring stocks spawn during the local spring. He also reported that length-specific fecundity is inversely related to latitude. Paulson and Smith (1977) compared fecundity–length relationships for some herring stocks between Washington and Peter the Great Bay. They found that fecundity decreased with increasing latitude and that average spawner length increased. Wespestad and Fried (1983) presented length-at-age and age-specific mortality rate data for various herring stocks between San Francisco and Kotezebue Sound in Alaska. They reported that fish from Bristol Bay were substantially longer than herring from stocks to the north or south. Age-specific mortality rates appeared to decrease with increasing latitude.

The goal of our study was to determine how latitude influenced the growth and reproductive characteristics of Pacific herring living in the Canadian Pacific Ocean and Beaufort Sea. We wanted to answer the following questions: (1) does size-at-age and do body size–length relationships differ, (2) do these populations allocate surplus energy differently with respect to growth and reproduction and as a function of age, (3) does seasonal gonad growth differ, (4) how comparable are gonad weight–, fecundity–, and egg weight – body size relationships, and (5) what is the adaptive significance of the similarities and differences?

Materials and Methods

We took advantage of the availability of data from two spring-spawning herring populations collected with the same gear type during the same year (1985) from two very different locations (Fig. 1). Age and size data (n = 1480) for herring from the Strait of Georgia came from routine biological sampling of commercial gillnet catches. Beaufort Sea herring data (n = 830) were from a feasibility study of roe herring fisheries

in the Arctic (A.H. Kristofferson and D.V. Gillman, unpublished results). Herring were gillnetted using 57-mm stretch mesh nylon monofilament gill nets.

Fish were sampled after thawing. Individual standard length (to the nearest 1 mm), total weight (to the nearest 1 g), and ovary weight (to the nearest 0.1 g) were measured. Scales were used to age fish. The herring collected in 1985 for fecundity estimates were the only Beaufort Sea fish aged that year. We ignored testes weights because the netting would force milt easily from running ripe males; therefore, testes weights would be inaccurate. We accepted ovary weights as ripe weights for fish collected no more than 4 d before spawning.

We estimated fecundity of Beaufort Sea herring (n = 137) using the procedure and equipment described in Tanasichuk and Ware (1987). We took random subsamples stratified by 10-mm length intervals. Wet egg weight (milligrams) was estimated as ripe ovarian weight × 1000/fecundity. We used the fecundity data for Strait of Georgia herring (n = 64) collected during a fecundity monitoring program (see Tanasichuk and Ware 1987).

Geometric mean regression (GMR) equations (Ricker 1973) were used to estimate length- and weight-at-age, body weight–standard length, and ripe ovary weight–, fecundity–, and egg weight–total weight regressions. These regressions were appropriate because size ranges of Strait of Georgia and Beaufort Sea herring differed considerably and, as for most biological data, assumptions of model I regressions (Sokal and Rohlf 1981) were not satisfied. We used equations given in Sokal and Rohlf to calculate statistics needed to compare regression lines because these procedures have not been developed for GMR yet. (These comparisons were intended to approximate analyses of covariance. We tested the significance of differences between regression coefficients using the methods that Sokal and Rohlf described. If slopes did not differ, then the pooled regression coefficient was estimated. The adjusted group mean, that is, the estimated y value at the grand mean x value for each regression line, was calculated and the significance of the difference tested.) Sokal and Rohlf considered using model I regression statistics, for example, sums of squares, reasonable for GMR tests. We used log-transformed data for length- and weight-at-age, body weight–standard length, and ripe ovary weight– and fecundity–total weight regressions. We calculated somatic weight as the difference between total weight and ripe ovarian weight estimated from the appropriate ripe ovary weight–total weight regression. Coefficients of determination and mean x and y values are given for all GMR equations so that the corresponding predictive regression equations can be calculated. Model I regression methods were appropriate for the maturation (GSI versus date) equations. We identified outliers to regressions as ordered pairs with Studentized residuals less than −2.5 or greater than 2.5 and leverage coefficients greater than $4/n$ (Sokal and Rohlf 1981). They were excluded from regression analyses. We also excluded fish shorter than 210 mm. Hay et al. (1986) reported that 57-mm-mesh nets begin catching herring effectively (at least 90% of the fish) only at fish lengths greater than 210 mm. Consequently, 5 and 443 of the Beaufort Sea and Strait of Georgia fish were excluded from regression analyses; 94% of the Strait of Georgia herring omitted were younger than 6 yr.

We calculated surplus energy, the amount of energy allocated to somatic growth and reproduction, following the procedure outlined by Ware (1985). It was estimated as the sum of the increase in somatic weight between consecutive ages and the ripe ovary weight, that is, $SE = (S_{x+1} - S_x) + Q_{x+1}$ where

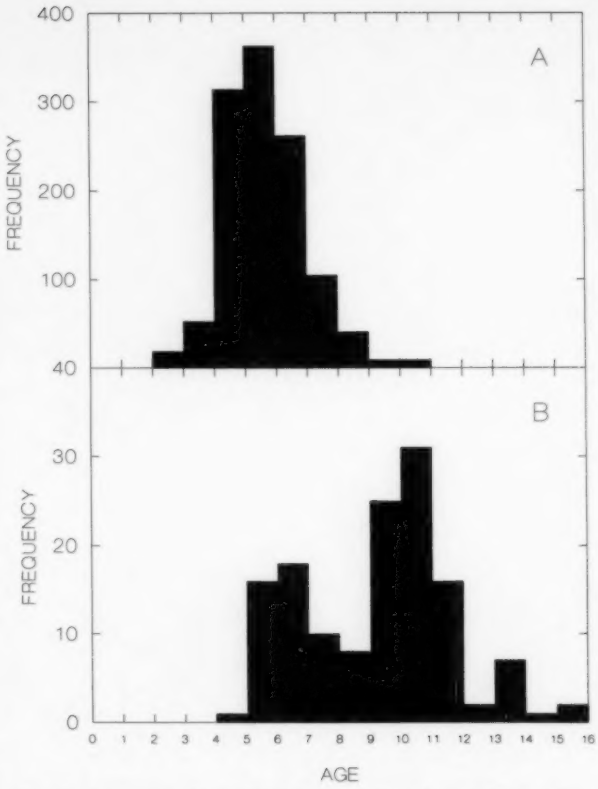

FIG. 2. Age composition of (A) Strait of Georgia and (B) Beaufort Sea herring. The histogram for herring collected in the Beaufort Sea in 1985 is based on a sample stratified by 10-mm length intervals.

SE = surplus energy (grams per year), S = somatic weight (grams), Q = ripe ovary weight (grams), and x = age. We calculated somatic weight as the difference between total weight and the estimated weight-specific ripe ovary weight. Ripe ovary weight was estimated from location-specific ripe ovary weight–total weight regressions.

We used data for Beaufort Sea herring collected over 1981–83 (Gillman and Kristofferson 1984) to examine seasonal ovarian growth and to calculate instantaneous total mortality rates using catch curves (Ricker 1975). These fish were captured using 64-mm-mesh gill nets and therefore could not be used for size-at-age comparisons. Numbers-at-age for Strait of Georgia fish came the routine sampling of commercial gillnet (57-mm mesh) catches over the same years. We assumed that this difference in gear would not influence the slope of the descending right limb. The instantaneous total mortality rate (Z) was estimated as the slope of the descending right limb of the catch curve. Frequency-at-age was the sum over 1981–83.

Results

Age and Growth

Age compositions differed markedly (Fig. 2). Herring from the Strait of Georgia were between 2 and 10 yr old. Beaufort Sea fish ranged in age from 4 to 15 yr.

Slopes of length- and weight-at-age relationships were significantly ($p < 0.0001$) greater in Beaufort Sea herring.

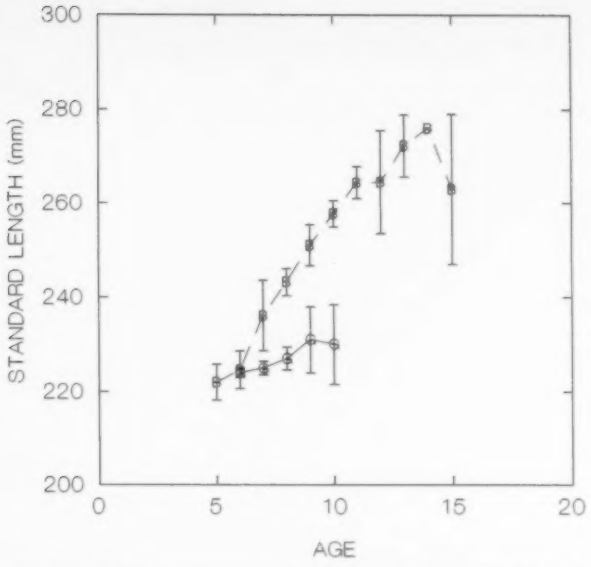

FIG. 3. Length-at-age (mean ± 2 SE) of Strait of Georgia (G) and Beaufort Sea (B) herring. Data for Strait of Georgia herring younger than 6 were excluded because of bias from gillnet selectivity.

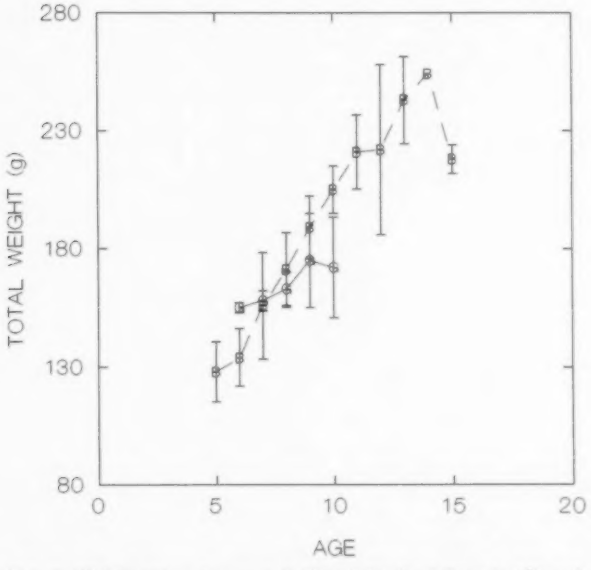

FIG. 4. Weight-at-age (mean ± 2 SE) of Strait of Georgia (G) and Beaufort Sea (B) herring. Data for Strait of Georgia herring younger than 6 were excluded because of bias from gillnet selectivity.

Length-at-age regressions were $L = 161A^{0.20}$ (mean age = 9.4, mean length = 252 mm, $R^2 = 0.93$, $p = 0.0001$) where L = standard length (millimetres) and A = age and $L = 200A^{0.06}$ (mean age = 8.8, mean length = 228 mm, $R^2 = 0.88$, $p = 0.02$) for Beaufort Sea and Strait of Georgia herring, respectively. We estimated weight-at-age regressions of $W = 46A^{0.64}$ where W = total weight (grams) (mean age = 9.4, mean weight = 193 g, $R^2 = 0.93$, $p < 0.0001$) and $W = 94A^{0.26}$ (mean age = 8.8, mean weight = 165 g, $R^2 = 0.88$, $p = 0.02$) for Beaufort Sea and Strait of Georgia fish, respectively. Mean length-at-age was equal at age 6; Beaufort Sea herring were significantly longer at older ages (Fig. 3). Mean total weight-at-age of Strait of Georgia herring was significantly greater at age 6, not significantly different through age 9, and significantly less at age 10 (Fig. 4).

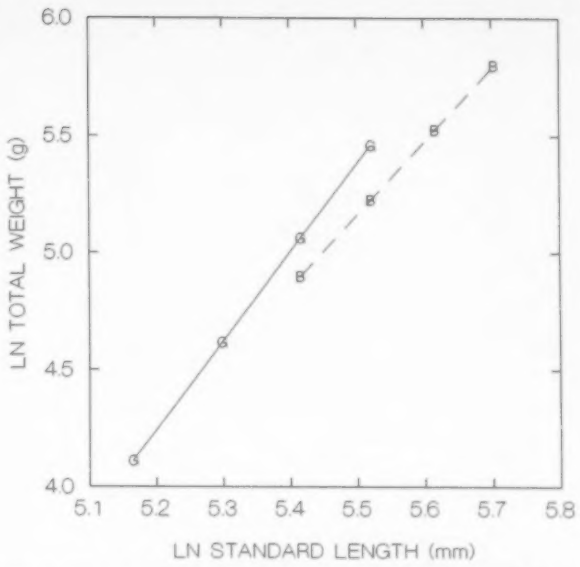

FIG. 5. Ln total weight–ln standard length relationships in Strait of Georgia (G) and Beaufort Sea (B) herring. Estimates plotted are over the ranges of observed lengths. Lines represent GMR equations.

The slopes of the total weight–standard length regressions differed significantly ($p < 0.01$) between locations (Fig. 5). We calculated regression equations of $\ln(W) = 3.78 \cdot \ln(L) - 15.41$ (mean length = 224 mm, mean weight = 155 g, $R^2 = 0.48$, $p < 0.0001$) and $\ln(W) = 3.14 \cdot \ln(L) - 12.11$ (mean length = 262 mm, mean weight = 216 g, $R^2 = 0.61$, $p < 0.0001$) for Strait of Georgia and Beaufort Sea herring, respectively.

We found no significant effect ($p > 0.10$) of location on somatic weight–standard length regressions. We calculated regression equations of $\ln(S) = 2.83 \cdot \ln(L) - 10.56$ (mean length = 224 mm, mean weight = 116 g, $R^2 = 0.47$, $p < 0.0001$) and $\ln(S) = 2.75 \cdot \ln(L) - 10.12$ (mean length = 262 mm, mean weight = 180 g, $R^2 = 0.61$, $p < 0.0001$) for Strait of Georgia and Beaufort Sea herring, respectively. The pooled regression equation was $\ln(S) = 2.69 \cdot \ln(L) - 9.74$ (mean length = 235 mm, mean weight = 141 g, $R^2 = 0.87$, $p < 0.0001$).

Reproduction

Location had no significant effect ($p > 0.75$) on the slopes of ripe ovary weight–total weight regressions (Fig. 6). Adjusted group mean ripe ovary weights differed significantly ($p < 0.001$). We calculated regression equations of $\ln(Q) = 1.74 \cdot \ln(W) - 5.06$ (mean weight = 158 g, mean Q = 42 g, $R^2 = 0.53$, $p < 0.0001$, $n = 446$) and $\ln(Q) = 1.65 \cdot \ln(W) - 5.30$ (mean weight = 252 g, mean Q = 46 g, $R^2 = 0.53$, $p < 0.0001$, $n = 42$) for Strait of Georgia and

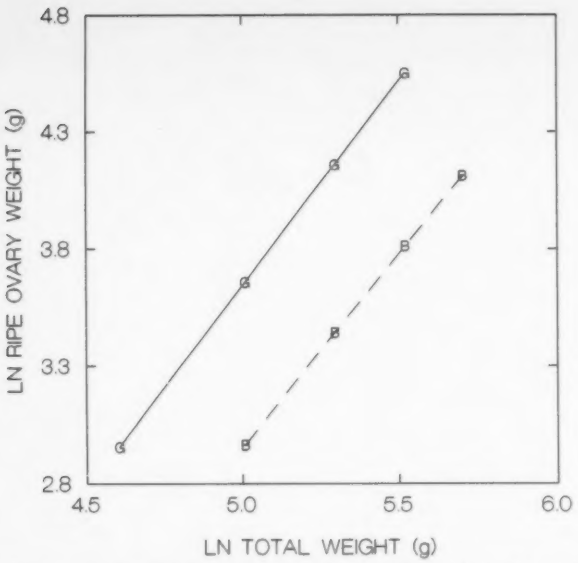

FIG. 6. Ln ripe ovary weight–ln total weight relationships in Strait of Georgia (G) and Beaufort Sea (B) herring. Estimates plotted are over the ranges of observed weights. Lines represent GMR equations.

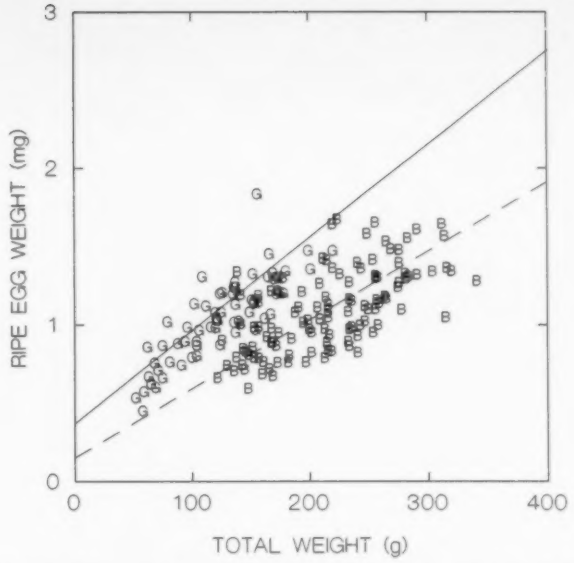

FIG. 8. Ripe egg weight–total weight relationships in Strait of Georgia (G) and Beaufort Sea (B) herring. Lines represent GMR equations.

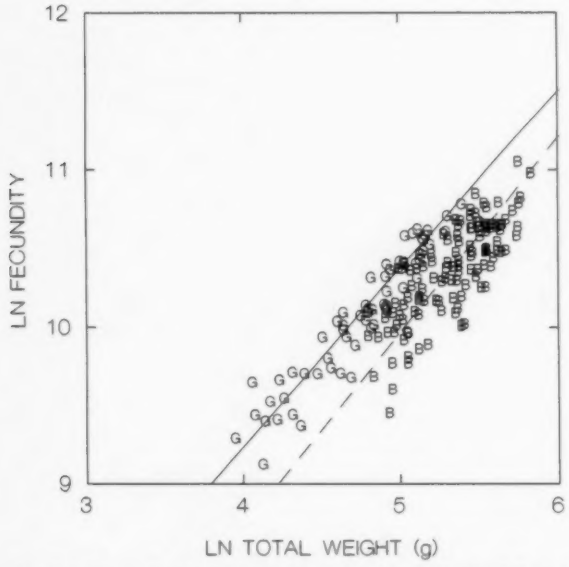

FIG. 7. Ln fecundity–ln total weight relationships in Strait of Georgia (G) and Beaufort Sea (B) herring. Lines represent GMR equations.

Beaufort Sea herring, respectively. Intercepts for regressions forced through the pooled slope (1.73) were −5.01 and −5.75 for Strait of Georgia and Beaufort Sea herring, respectively. Weight-specific Q's for Strait of Georgia herring were 2.1 times greater than those for Beaufort Sea fish.

Intercepts of fecundity (F)–total weight regressions differed significantly ($p < 0.001$) (Fig. 7). Regression equations were $\ln(F) = 1.24 \cdot \ln(W) + 3.76$ (mean weight = 206 g, mean fecundity = 31 779 eggs, $R^2 = 0.65$, $p < 0.0001$) for Beaufort Sea herring and $\ln(F) = 1.13 \cdot \ln(W) + 4.69$ (mean weight = 118 g, mean fecundity = 23 882 eggs, $R^2 = 0.89$, $p < 0.0001$) for Strait of Georgia herring. Intercepts estimated for the pooled slope (1.24) were 3.97 and 4.36 for Beaufort Sea and Strait of Georgia herring, respectively. Weight-specific fecundities were 1.5 times greater for Strait of Georgia fish.

We found a significant relationship between female weight and ripe egg weight for each stock (Fig. 8). GMR slopes differed insignificantly ($0.10 < p < 0.25$). The regressions were $E = 0.005 \cdot W - 0.10$ (mean weight = 237 g, mean egg weight = 1.09 mg, $R^2 = 0.43$, $p < 0.0001$ where E = ripe egg weight (milligrams)) and $E = 0.006 \cdot W + 0.37$ (mean weight = 136 g, mean egg weight = 1.19 mg, $R^2 = 0.70$, $p < 0.0001$) for Beaufort Sea and Strait of Georgia females, respectively. The intercepts we estimated for the pooled mean slope (0.005) of −0.10 and 0.37 for Beaufort Sea and Strait of Georgia herring, respectively, differed significantly ($p < 0.001$). Weight-specific ripe egg weights were 1.4 times greater for Strait of Georgia herring.

Variations in female gonadosomatic index (GSI) over time for Beaufort Sea herring collected during 1981–83 are presented in Fig. 9. We found no significant ($p > 0.10$) differences in regression slopes. GSI increased linearly over the last 56 d and at a weighted pooled rate of 0.18% GSI·d^{-1}. Results of an analysis of covariance showed that the adjusted group mean GSI was significantly ($p < 0.01$) greater in 1981.

Surplus Energy

Surplus energy schedules for age 6–10 herring are presented in Table 1. Beaufort Sea herring accumulated surplus energy at a rate averaging 40 g·yr^{-1}, independent of fish size. The proportion of surplus energy assigned to reproduction increased with age (or size) and represented 0.54 and 0.73 of the total surplus energy for age 6 and 10 fish, respectively. The rate of surplus energy accumulation for Strait of Georgia herring averaged 49 g·yr^{-1}, also independent of fish size. Age 6 and older fish used most (mean = 0.95) of their surplus energy for ovar-

TABLE 1. Surplus energy (SE) schedules for Beaufort Sea and northern Strait of Georgia herring.

Age (yr)	W (g)	Q (g)	S (g)	SE (g)	Proportion SE: Growth	Proportion SE: Reproduction
			Beaufort Sea			
6	134	16	118			
				37	0.46	0.54
7	155	20	135			
				35	0.31	0.69
8	170	24	146			
				43	0.35	0.65
9	189	28	161			
				45	0.27	0.73
10	206	33	173			
			Strait of Georgia			
6	155	41	114			
				44	0.05	0.95
7	158	42	116			
				47	0.04	0.96
8	163	45	118			
				57	0.11	0.89
9	175	51	124			
				48	0.00	1.00
10	172	48	124			

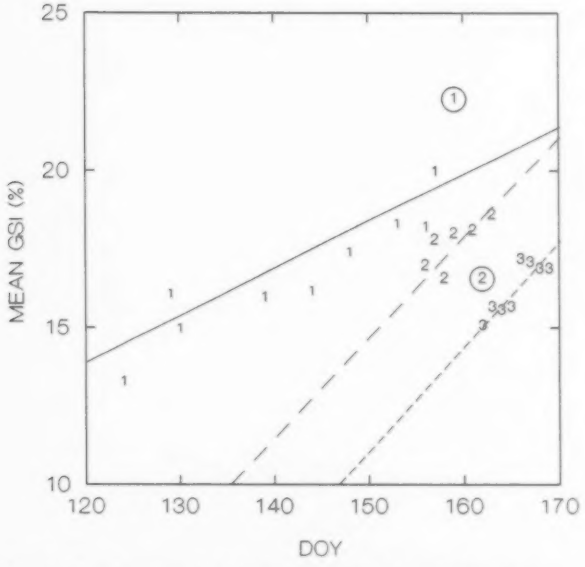

FIG. 9. Ovarian growth trajectories for Beaufort Sea herring over 1981–83. Encircled symbols identify outliers that were excluded from the regressions. Lines represent model I (predictive regressions). 1981 (1): GSI = 0.15·DOY − 4.45, $p < 0.0001$, $R^2 = 0.86$; 1982 (2): GSI = 0.22·DOY − 16.92, $p = 0.08$, $R^2 = 0.58$; 1983 (3): GSI = 0.29·DOY − 32.19, $p = 0.006$, $R^2 = 0.75$.

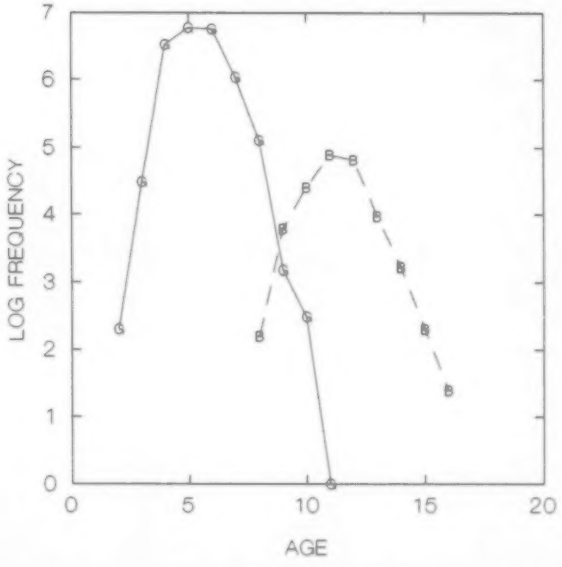

FIG. 10. Catch curves for Strait of Georgia (G) and Beaufort Sea (B) herring. Frequency is the number of fish-at-age captured over 1981–83.

ian development. Surplus energy accumulation, as a proportion of the initial somatic weight, was 1.45 times greater in Strait of Georgia herring (0.42) than in Beaufort Sea herring (0.29).

Mortality Rates

We calculated instantaneous total mortality rates using the catch curves shown in Fig. 10. Slopes of the catch curves for age 7–11 Strait of Georgia herring and age 12–16 Beaufort Sea herring were −1.51 and −0.86, respectively.

Discussion

Herring from the Strait of Georgia and the Beaufort Sea showed differences and, surprisingly, similarities in their growth and reproductive characteristics. We expected Beaufort Sea fish to have lower annual growth rates and to be in poorer condition because of anticipated lower sea temperatures and secondary productivity. We had no notions about differences in surplus energy accumulation and allocation. We thought that egg size would be greater and maturation substantially slower because of a colder ocean.

We found that Beaufort Sea adults grew more rapidly in length but not in weight. Such changes in length- and weight-at-age suggest that condition for Strait of Georgia herring would be progressively greater than for Beaufort Sea herring as individuals age as adults. Hay (1985) stated that a cursory comparison of length–weight data from different latitudes suggested that, for a given length, herring tended to be heavier in the south. He thought that there may be problems with the comparison of length–weight data collected by different investigators on different stocks varying in their degree of maturation. Our data are for fish collected at the same time in the season cycle, that is, at spawning, and this tendency persisted.

We found that total weight–standard length relationships differed because of the amount of surplus energy accumulated and allocated to reproduction. Location had no significant effect on somatic weight–standard length relationships. Such comparisons have not been previously reported. They imply a common strategy, between these two groups at least, resulting in the allocation of a length-specific proportion of surplus energy to somatic growth. This could be to provide the somatic framework required to support at least a minimum amount of reproductive product.

We found that adult mortality rates were substantially greater for Strait of Georgia herring and suggest that differences in surplus energy strategy are a response to this. Instantaneous total mortality rates were 1.8 times higher. We also calculated natural mortality rates for Strait of Georgia herring because Beaufort Sea herring are not harvested and it is unknown how quickly the Strait of Georgia herring population could adapt to the additional mortality from fishing. Fishing mortality was estimated as 0.43 from the exploitation rate (m) using $m = 1 - e^{-F}$ (Ricker 1975) where m is the mean exploitation rate (0.35) for all year-classes of fish used in this study. (We estimated the exploitation rate using numbers-at-age derived from the escapement model for British Columbia herring stock assessment (Schweigert and Stocker 1988)). The instantaneous natural mortality rate (M) for Strait of Georgia herring was 1.08 (1.51 − 0.43) which was 1.26 times that for Beaufort Sea herring. Our M's are substantially higher than that reported by Beverton (1963). He stated that Tester (1955) estimated $M = 0.45$. However, an M of 0.45 was only for age 4 fish, and Tester showed that it increased progressively up to 1.14 for age 7 herring.

Strait of Georgia herring accumulated surplus energy 45% (0.42/0.29) more rapidly. The slower accumulation of surplus energy by Beaufort Sea herring, at least through the reproductive phase of their life, seems to be a reasonable approach to a situation where predation pressure is relatively low. Maximum potential fecundity for these fish is realized by accumulating surplus energy more conservatively.

Our results show large differences in surplus energy partitioning between growth and reproduction. Strait of Georgia herring between ages 6 and 10 allocate virtually all (0.95) their surplus energy to reproduction. In contrast, the proportion of surplus energy used for reproduction increased from 0.54 to 0.73 for age 6 and 10 Beaufort Sea herring, respectively. However, data presented in Ware (1985) showed that the proportion of surplus energy assigned to reproduction increases from 0.57 to 0.83 for ages 3–6 for a British Columbia herring stock. Therefore, both stocks exhibit the same trend of increasing surplus energy allocated to reproduction once the fish mature, but this begins at age 3 for Strait of Georgia herring and age 6 for Beaufort Sea herring.

Ripe ovary weight is a constant function of fish size for both stocks but is, on a total-weight-specific basis, twice as great in Strait of Georgia herring. The constancy of the relationship within each group could reflect, as suggested by Ware (1984), an approach that maximizes fitness when there is a survival cost associated with reproduction. Strait of Georgia herring accept the high cost because of high adult mortality. On the other hand, Beaufort Sea herring evolved a lower cost because a high reproductive rate is unnecessary due to a lower predation pressure. Their survival cost could be a consequence of food limitation. Lawrence et al. (1984) showed that herring in the Beaufort Sea have a diverse diet which consists of plankton and benthos. This implies that the supply of macrozooplankton as food is inadequate. In addition, length- and weight-at-age relationships show that Beaufort Sea herring are in poorer condition as adults.

Age composition differences between the two groups, based on the catch curves, suggested differences in maximum age. Our results suggest that typical maximum ages are 11 and 16 for Strait of Georgia and Beaufort Sea herring, respectively. Hay (1985) and Riske (1960) reported age-at-maturity of 3 and 6 for British Columbian and Beaufort Sea herring, respectively. Age-at-first-maturity appears to support Beverton's (1963) observation that herring reproduce for about 0.68 of their life span. It also supports his speculation that, in relatively short-lived species such as Pacific herring, maturation may be more closely related to longevity than growth is. The older age-at-maturity for Beaufort Sea herring appears to reflect the lower adult mortality rate. Murphy (1968) felt that high or variable adult mortality could lower age-at-maturity. This certainly appears to have occurred in Strait of Georgia herring. These fish must face an average of 300 000 tonnes of predator biomass over summer (D.M. Ware and R.W. Tanasichuk, unpublished results).

Gonad growth characteristics are similar between both groups even though the oceanographic conditions they mature under differ substantially. In both groups, males initially mature more rapidly. Ware and Tanasichuk (1989) developed models of gonad growth that showed this for British Columbia herring. Riske (1960) stated that for herring collected near Tuktoyaktuk in September 1958, males were running ripe with a mean GSI of 15.8% whereas females were half full of small whitish eggs and had a mean GSI of 6.1%. Lawrence et al. (1984) reported that mean GSI of female herring was 4.7 and 13.7% in September 1980 and March 1981, respectively. Maturation rates may be even higher in Beaufort Sea males because Ware and Tanasichuk (1989) showed that Strait of Georgia males would have GSI's averaging about 4% in September. Ware and Tanasichuk (1989) reported that over the last month before spawning, female British Columbia herring mature at an average rate (K) of 0.32% GSI·d^{-1}. This occurs at temperatures ranging between 6.5 and 9.8°C (Haegele and Schweigert 1985). We estimated an average K of 0.18 for Beaufort Sea herring.

However, these fish mature under the ice between October and spawning in June–July at sea temperatures ranging between −2 and 0°C (Hopky et al. 1986). We estimated a Q_{10} of 1.9 for K using mean temperatures for the observed ranges (8.2 and −1.0°C for Strait of Georgia and Beaufort Sea herring, respectively). This is reasonably close to the Q_{10} of 2.4 estimated by Ware and Tanasichuk (1989) and suggests that ovarian growth is similar between the stocks, at least with respect to the influence of sea temperature.

Size-specific fecundity differences are due to size-specific ripe ovary weight and egg weight effects. Ripe ovary weights were 110% greater in Strait of Georgia herring and size-specific ripe egg weights were about 30% smaller in Beaufort Sea herring. Tanasichuk and Ware (1987) showed there is an adaptive trade-off between fecundity and egg size both between and within stocks of British Columbia herring. They suggested that egg weight should decline with increasing temperature to offset an anticipated increase in predation pressure during the larval phase. Beaufort Sea herring appear to exemplify this. Sea temperatures during the larval phase would be about 12°C (Shields 1985) and 9.5°C (Alderdice and Hourston 1985) for Beaufort Sea and Strait of Georgia herring, respectively. Consequently, weight-specific egg size should be smaller in Beaufort Sea herring to anticipate a greater larval predation pressure.

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Sites of Methyl Mercury Production in Remote Canadian Shield Lakes[1]

P.S. Ramlal and C.A. Kelly

Department of Microbiology, University of Manitoba, Winnipeg, Man., Canada R3T 2N2

and J.W.M. Rudd,[2] and A. Furutani

Department of Fisheries and Oceans, Central and Arctic Region, Freshwater Institute, 501 University Crescent, Winnipeg, Man., Canada R3T 2N6

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Methylation and demethylation of mercury were studied in sediments and surface waters of several remote lakes on the Canadian Shield. Radiochemical assays of mercury methylating activity, which peaked during summer, were 20–40 times faster in epilimnetic than in hypolimnetic sediments. Demethylation rates were usually highest during winter and in hypolimnetic sediments. Epilimnetic sediments were capable of producing methyl mercury 20–40 times faster than hypolimnetic sediments sampled at the same time, with methylating activity peaking during the warm summer months. Because of the opposite pattern of methylating and demethylating activity and because epilimnetic sediments often constitute most of the surface area of these lakes, most of the net methylation (*M/D*) occurred in the epilimnion of the lakes during summer. Mercury methylation rates were not related to average sediment mercury concentrations, or directly to rates of microbial activity (decomposition) in sediments. It appeared that increasing temperature was an important controlling factor that stimulated methylation but retarded demethylation. Specific methylation rates, *M/D*, and the measured concentration of methyl mercury in the surface waters of the lakes all peaked during midsummer, suggesting that the radiochemical assays used reflected changes in the relative activities of natural methylating and demethylating microorganisms.

La méthylation et la déméthylation du mercure ont été étudiées dans les sédiments et les eaux superficielles de plusieurs lacs isolés du Bouclier canadien. Les essais radiochimiques sur l'activité de méthylation du mercure, qui atteint un maximum pendant l'été, montraient une activité de 20 à 40 fois plus rapide dans les sédiments épilimniques que dans les sédiments hypolimniques. Le taux de déméthylation était généralement au maximum pendant l'hiver et dans les sédiments hypolimniques. Les sédiments épilimniques pouvaient produire du méthylmercure 20–40 fois plus vite que les sédiments hypolimniques échantillonnés au même moment, et l'activité de méthylation était au maximum pendant les chauds mois d'été. À cause du régime opposé de méthylation et de déméthylation, et du fait que les sédiments épilimniques constituent souvent la plus grande partie de la superficie de ces lacs, la plus grande partie de la méthylation nette (*M/D*) se produisait dans l'épilimnion des lacs pendant l'été. Les taux de méthylation du mercure n'étaient pas liés aux concentrations moyennes de mercure dans les sédiments, ni directement à l'activité microbienne (décomposition) dans les sédiments. Il semble que la hausse de température était un facteur important qui stimulait la méthylation mais retardait la déméthylation. Les taux spécifiques de méthylation, le rapport *M/D* et la concentration mesurée de méthylmercure dans les eaux superficielles des lacs marquaient tous un maximum au milieu de l'été, ce qui semble indiquer que les essais radiochimiques faisaient apparaître des changements dans les activités relatives des micro-organismes qui sont naturellement responsables de la méthylation et de la déméthylation.

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Mercury contamination of fish has been a topic of concern for many years. Most of these contamination problems are caused by anthropogenic disturbances such as point-source pollution (D'Itri 1972; Armstrong and Hamilton 1973; Rudd et al. 1983), atmospheric acidification of lakes (Jernelov et al. 1976), or construction of hydroelectric reservoirs (Bodaly et al. 1984; Hecky et al. 1991). In addition to these anthropogenic problems, mercury-contaminated fish have been found in certain nonacidified lakes that are remote from human disturbance and have no point source of mercury. To improve our basic understanding of this type of problem, we studied mercury biogeochemistry in a series of lakes located in a remote, unaltered region of northwestern Ontario. The series of lakes, known as the Northwest Ontario Lake Size Series (NOLSS; Fee and Hecky 1992), are being studied to determine the effects of lake size on various physical and biological processes. This type of basic research is necesary not only to understand mercury contamination of fish in remote lakes, but also to understand changes in mercury biogeochemistry following anthropogenic disturbances.

Our research had the following specific goals: (1) to compare specific rates of net methyl mercury production between epilimnetic and hypolimnetic sediments within a lake, (2) to compare specific rates of net methyl mercury production among

[1]This is one of a series of NOLSS papers dealing with the lake size series of northwestern Ontario.

[2]Author to whom reprint requests should be directed.

TABLE 1. Physical and chemical data for NOLSS study lakes, northwestern Ontario (51°N, 94°W).

Lake	Surface area (ha)[a]	Drainage area (ha)[a]	τ (yr)[a]	Z_{max} (m)[a]	$\bar{Z}$ (m)[a]	V_c/A_c[b] (m)	k_{25} ($\mu S \cdot cm^{-1}$)[a]	pH	DOC ($\mu mol \cdot L^{-1}$)	Alkalinity ($\mu eq \cdot L^{-1}$)	SO_4^{2-} ($\mu mol \cdot L^{-1}$)	Cl^- ($\mu mol \cdot L^{-1}$)	Chl *a* ($\mu g \cdot L^{-1}$)
Green	89	234	8.6	19	7.7	12.5	28	7.5	510	170	24	5.6	1.7
Linge	706	2 980	6.7	23	8.4	9.1	30	7.3	630	210	22	2.8	2.8
Sydney	5 748	46 400	9.1	73	20.0	20.0	41	8.0	520	290	30	8.5	2.9
Trout	34 690	71 800	17.5	49	13.6	14.3	62	8.0	310	570	26	5.6	1.8

[a]Physical data from Fee and Hecky (1992).
[b]Ratio of epilimnion volume to epilimnion area during summer. Physical data from Fee and Hecky (1989).

TABLE 2. Average bulk densities, mercury concentrations, and organic carbon concentrations of sediment sampling sites in the study lakes.

Lake	Depth (m)	*n*	Bulk density ($mg \cdot mL^{-1}$)	Hg ($pmol \cdot mL^{-1}$)	Organic C ($\mu mol \cdot L^{-1}$)
Green	2	5	570 (340–700)	16 (9–32)	0.56 (0.53–0.70)
	12	4	40 (19–65)	15 (7–25)	0.41 (0.02–0.64)
	18	3	28 (16–46)	17 (10–25)	0.38 (0.02–0.58)
Linge	3	2	730 (720–740)	23 (17–29)	0.40 (0.40–0.40)
Sydney	2	2	900 (860–970)	13 (0.0–25)	0.30 (0.24–0.36)
	46	2	150 (120–180)	36 (25–48)	0.53 (0.41–0.64)
Trout					
BB	3	5	980 (780–1100)	8 (0.0–17)	0.34 (0.30–0.37)
PB	2	4	130 (70–120)	15 (10–21)	0.65 (0.49–0.86)
	43	2	93 (85–100)	21 (18–24)	0.45 (0.41–0.48)

lakes of different size, (3) to compare rates of sediment respiration to rates of net mercury methylation in epilimnetic and hypolimnetic sediments, and (4) to determine whether radioisotopic assays of specific rates of mercury methylation and demethylation are related to in situ changes in methyl mercury concentrations.

Methods and Materials

Study Sites

Studies were conducted on material collected from the NOLSS lakes in the Red Lake District of northwestern Ontario (51°N, 94°W). The NOLSS lakes are a group of undisturbed Canadian Shield lakes varying in size over nearly three orders of magnitude (Fee and Hecky 1992). The lakes were chosen with size as the primary variable. Other parameters such as water renewal time, conductivity, and water chemistry were chosen to be as similar as possible (Table 1). During the summer, they are only accessible by floatplane and are uninhabited except for fly-in fishing lodges on the two largest lakes.

In Trout Lake, the largest study lake, two epilimnetic sites, Pleasant Bay (PB) and Beach Bay (BB), representing extremes in sediment porosity were cored. One epilimnetic site was cored in each of Sydney, Green, and Linge lakes. In 1988, hypolimnetic cores were taken from 12 m depth in Green Lake, 43 m in Trout Lake, and about 46 m in Sydney Lake. In Green Lake in 1989, hypolimnetic cores were taken at both 12 and 18 m; the hypolimnion remained oxic throughout the year with detectable oxygen remaining at Z_{max} (18 m) at the end of summer stratification.

A range of sediment types were used in this study (Table 2). Hypolimnetic sediments were of high porosity with a dry bulk density (sediment dry weight/volume) of 28–150 $mg \cdot mL^{-1}$, while epilimnetic sediments had low porosities and high bulk densities, 570–980 $mg \cdot mL^{-1}$, except for one site in Trout Lake which had a high porosity. Organic carbon content ranged from 0.02 to 0.86 $\mu mol \cdot mL^{-1}$. Mean total mercury content of sediments on a volumetric basis did not systematically vary with lake depth or lake size (Table 2). Cores were taken from epilimnetic sites (2–3 m deep) by snorkel diver using 5.0-cm-diameter Plexiglas core tubes. Hypolimnetic sediments were sampled using a KB-corer (Wildco). Water temperature was measured using a telethermometer (Flett Research Inc.).

During transport, cores were kept close to in situ temperature (±2°C). Within 3–4 h of sampling the intact cores were incubated to measure respiration rates. Within 1—2 d of these measurements the cores were transported to the laboratory in Winnipeg for mercury methylation and demethylation measurements.

Sediment Respiration Measurements

Cores were equilibrated for 1 h at in situ temperature, with top stoppers removed, prior to beginning incubations. Subsamples of the overlying water in the core were taken for dissolved inorganic carbon (DIC) analysis. After sampling the surface water, the cores were sealed with no air space. In situ water movement was simulated using a magnetic stir bar that was suspended from the top stopper and agitated by an intermittent magnetic field (M. Holoka, Freshwater Institute, personal communication). This degree of agitation resulted in similar microprofiles of oxygen concentrations across the sediment–water interface as measured in situ in these lakes (J.-P.R.A. Sweerts, Vijverhof Laboratory of the Limnological Institute, Nieuwersluis, The Netherlands, personal communication). Cores were incubated at in situ temperature, usually for about 12 h for epilimnetic cores and 24 h for hypolimnetic cores, and sampled again for DIC at the end. DIC was measured on 20-mL subsamples of the water overlying the sediment. The 20-mL aliquot was injected into an evacuated 37-mL serum bottle and preserved/acidified with 1 mL of 2 M H_2SO_4. The serum bottle was shaken and the concentration of carbon dioxide in

the headspace measured using thermal conductivity gas chromatography (Hewlett Packard model 5890A) equipped with a Haye-Sep column. Methane release from these sediments was small (<5%) relative to carbon dioxide. Thus, sediment respiration was considered to be equal to DIC release.

Measurements of Specific Rates of Mercury Methylation and Demethylation

For each site the top 2 cm from three cores were removed, combined, and distributed into the methylation and demethylation incubation bottles. The diameter of the bottles was such that the sediment depth of 2 cm (15 mL) was maintained. Each pooled sample was split into two trial and one killed blank for each methylation and demethylation assay. Five millilitres of lake water was added to each subsample. The remaining sediment was used to estimate the dry weight of the original sediment and for chemical analysis. Methylation samples were spiked with 74 kBq of $^{203}HgCl_2$ (specific activity = 37 kBq·μg Hg^{-1}). Demethylation samples were spiked with 0.74 kBq of $^{14}CH_3$ HgI (specific activity = 733 MBq·mmol^{-1}). Samples were incubated for 12 h at in situ temperature and then killed with 2 mL of 4 M HCl. The alkylated ^{203}Hg was assayed using the organic extraction method of Furutani and Rudd (1980). The amount of ^{14}C released from the labelled methyl mercury was measured as described by Ramlal et al. (1986). Specific rates were expressed as micromoles of mercury methylated or demethylated per square metre per day. Results were also expressed as a ratio of these two rates (*M/D*). Water column methylation and demethylation were measured as described in Xun et al. (1987).

Sediment and Water Analyses

Total mercury in the sediment was measured on dried sediments by atomic absorption (Armstrong and Uthe 1971). Organic carbon concentration was estimated by combustion of the dried sample (Stainton et al. 1977).

Water samples for measurement of methyl mercury were collected in teflon bottles using ultraclean techniques (N. Bloom, Frontier Geoscience, Seattle, WA, personal communication). Methyl mercury concentrations in water were analyzed by Brooks-Rand, Ltd. (Seattle, WA) using atomic fluorescence as described in Bloom and Fitzgerald (1988) and Bloom (1989).

Results

In the spring of 1988, the lakes warmed quickly (Fee and Hecky 1992). Consequently, maximum epilimnetic water temperatures were observed in June rather than in midsummer (Fig. 1). This pattern was most pronounced in the sediment temperature of the smallest lake, Green Lake, although it was also evident at a shallow site in Trout Lake, the largest lake. In 1989, temperature followed a more typical pattern with maximum temperatures of epilimnetic sites occurring in July and early August (Fig. 1). Hypolimnetic temperatures in Green and Sydney lakes did not change much throughout the open water season; however, the hypolimnetic temperature of Trout Lake gradually warmed during the open-water period of 1988 (Fig. 1).

In each of the lakes, respiration in epilimnetic sediments (i.e., production of DIC) and water temperature exhibited similar patterns (Fig. 1 and 2). In 1988, when the lakes warmed early,

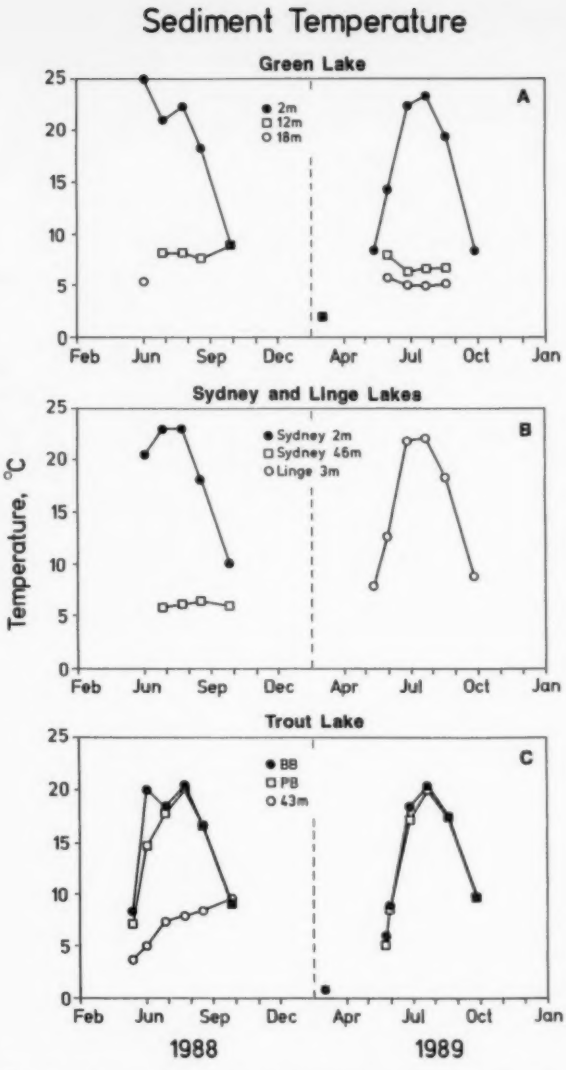

FIG. 1. In situ surface sediment temperature in (A) Green Lake, (B) Linge and Sydney lakes, and (C) Trout Lake.

respiration in epilimnetic sediments peaked earlier in the year than they did in 1989 (Fig. 2).

During the summer, respiration rates in the shallow sediments exceeded that of the hypolimnetic sediments by two- to fourfold (Fig. 2). However, near the end of the open-water period in October, there was little difference between epilimnetic and hypolimnetic respiration rates. This was also the case in early spring measurements of respiration at the epilimnetic and hypolimnetic sites in Trout Lake. During the winter, respiration of the hypolimnetic sediments of Green Lake exceeded the epilimnetic sediment respiration (Fig. 2).

Summer rates of specific methylation in epilimnetic sediments exceeded the rates in hypolimnetic sediments, by up to orders of magnitude (Fig. 3). For example, in Green Lake during the summers of 1988 and 1989, peak specific methylation rates in the epilimnetic sediments were 0.17 and

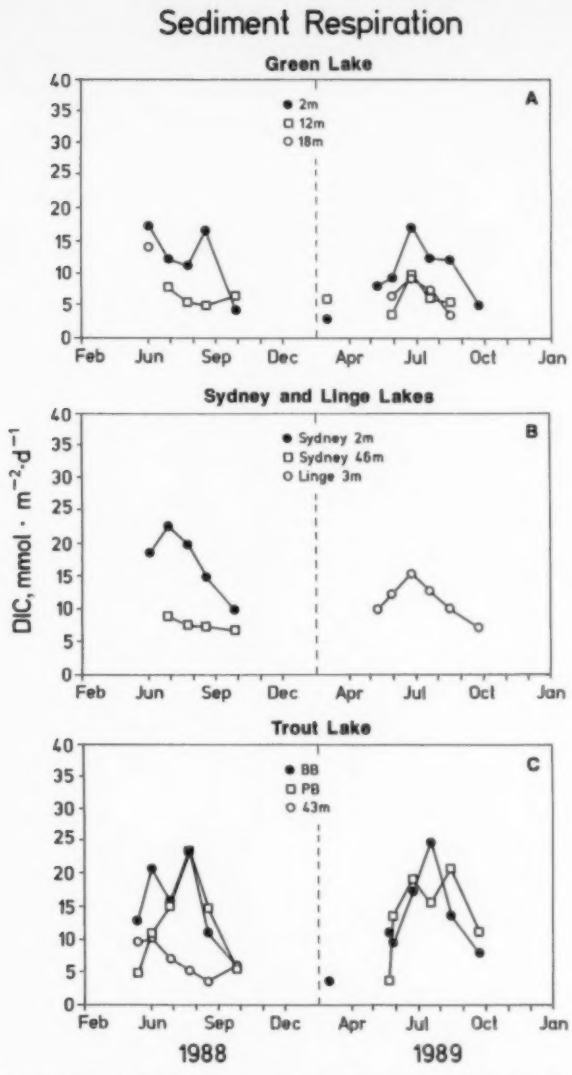

FIG. 2. Microbial respiration rates in epilimnetic and hypolimnetic sediments of (A) Green Lake, (B) Linge and Sydney lakes, and (C) Trout Lake.

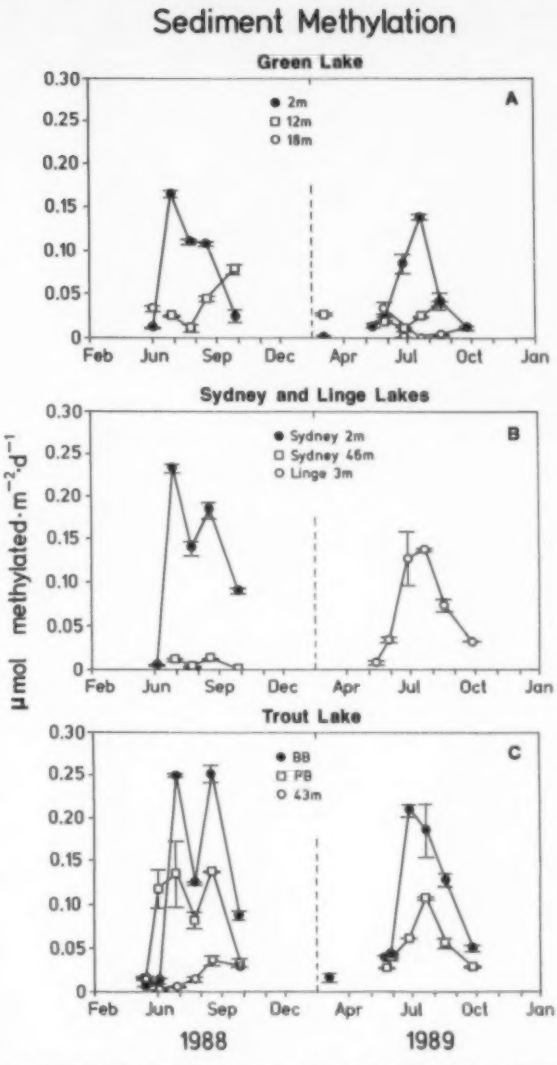

FIG. 3. Specific rates of mercury methylation in epilimnetic and hypolimnetic sediments of (A) Green Lake, (B) Linge and Sydney lakes, and (C) Trout Lake, showing a predominance of methylation occurring in epilimnetic sediments during summer.

0.13 $\mu mol \cdot m^{-2} \cdot d^{-1}$, respectively, whereas peak methylation rates in the hypolimnion were 0.04–0.08 $\mu mol \cdot m^{-2} \cdot d^{-1}$. Similar differences were found in epilimnetic/hypolimnetic comparisons of Trout, Sydney, and Linge lakes (Fig. 3).

In contrast with methylation, epilimnetic demethylation rates were similar to or lower than hypolimnetic rates (Fig. 4*a*). Also, in all lakes, demethylation rates peaked early in the summer and then declined more rapidly than did methylation (Fig. 3 and 4).

The *M/D* ratio peaked in either July or August at all epilimnetic sites (Fig. 5). Hypolimnetic *M/D* was much lower than epilimnetic *M/D* during summer, but this relationship was reversed during the winter (Fig. 5*a*).

There were greater differences in methylation and demethylation rates and *M/D* within a single lake, comparing epilimnetic with hypolimnetic sites, than from lake to lake when comparing all epilimnetic sites, or all hypolimnetic sites (Fig. 3, 4, and 5).

In the epilimnetic water of the NOLSS lakes, net methylation (*M/D*) varied with lake size (Fig. 6). The highest *M/D* ratios were found in Green lake, the smallest lake in the size series. Linge Lake, a medium-sized lake (Table 1), had lower *M/D* ratios whereas *M/D* was lowest at two epilimnetic water sampling sites in Trout Lake, the largest lake. The *M/D* peaked in all lakes during midsummer.

Methyl mercury concentrations were measured in epilimnetic water from Green, Linge, and Trout lakes during the open-water period of 1989 (Fig. 7). These values ranged from 0.01 to 0.13 $ng \cdot L^{-1}$. Peak methyl mercury concentrations occurred during midsummer and decreased as the water temperature

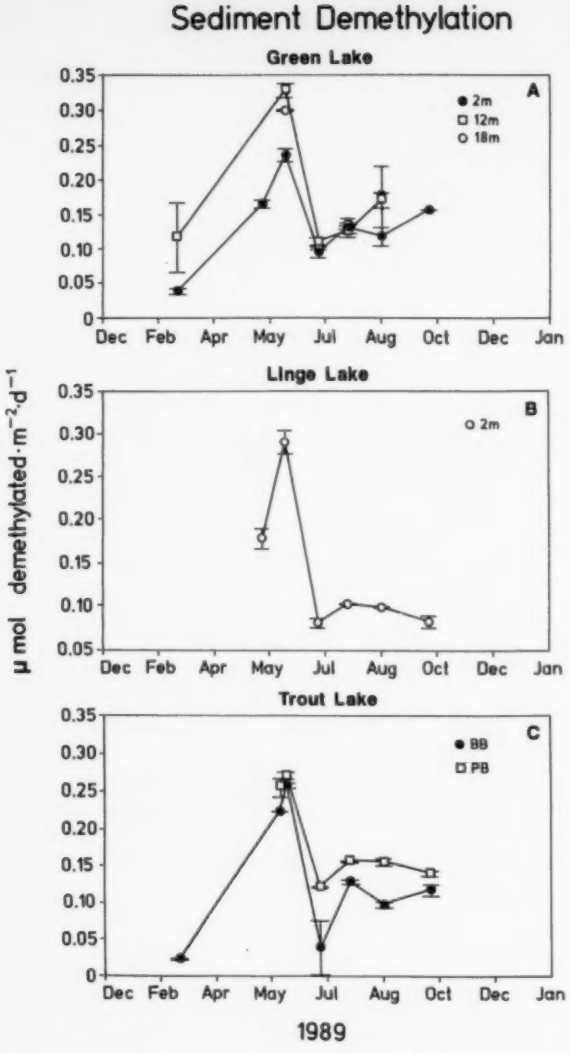

FIG. 4. Specific rates of mercury demethylation in epilimnetic and hypolimnetic sediments of (A) Green Lake and in epilimnetic sediments of (B) Linge Lake and (C) Trout Lake. Hypolimnetic demethylation rates exceeded epilimnetic rates during the ice-free season.

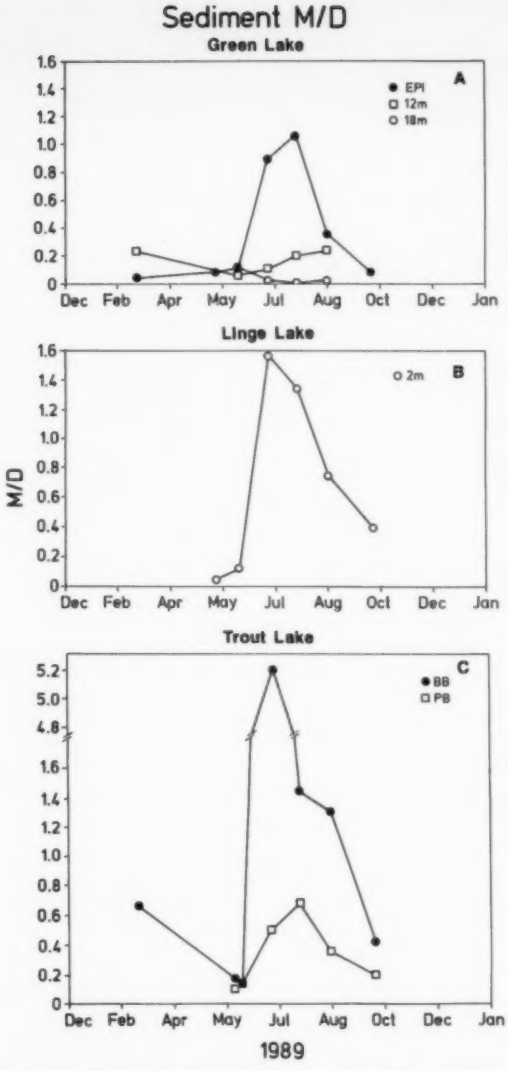

FIG. 5. *M/D* in epilimnetic and hypolimnetic sediments of (A) Green Lake and in epilimnetic sediments of (B) Linge and Sydney lakes and (C) Trout Lake. *M/D* in epilimnetic sites exceeded *M/D* in hypolimnetic sites during the ice-free season.

decreased. These trends in concentration corresponded to trends in specific methylation rates and *M/D* (Fig. 7). Although we could not do many replicate analyses on samples taken from these study lakes, samples taken in replicate from lakes in the Experimental Lakes Area using the same methods had a mean percent difference of 17.7 ± 15.9% (n = 21; J.W.M. Rudd, unpublished data).

Discussion

Comparisons of Radiochemical Assays and Trends of in situ Methyl Mercury Concentrations

One of the objectives of this study was to compare seasonal changes in surface-water methyl mercury concentrations with changes in specific rates of epilimnetic mercury methylation, demethylation, and *M/D* in water and sediments. The radiochemical methods for methylation and demethylation have been used for a number of years (e.g., Callister and Winfrey 1986; Ramlal et al. 1986; Xun et al. 1987; Hecky et al. 1991). However, we have not been able to compare specific rates of methylation and *M/D* with changes in in situ methyl mercury concentration until recently, after improvements in analytical detection (Bloom 1989) allowed measurement of the low-level methyl mercury concentrations found in natural lakes. The results of this study showed that the timing of midsummer maxima in methylation rates and *M/D* in both water and sediment (Fig. 6 and 7) corresponded to maxima of in situ surface-water methyl mercury concentrations (Fig. 7) in all

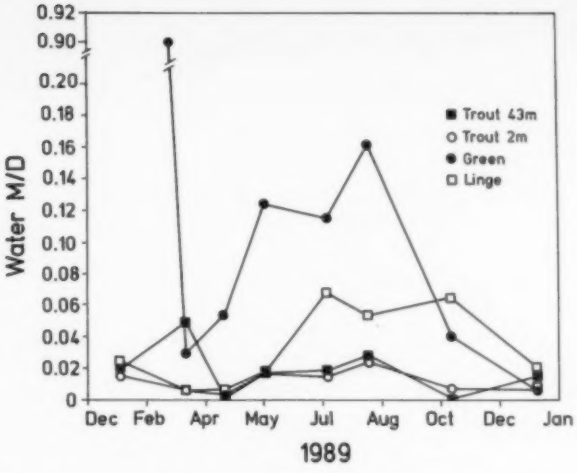

FIG. 6. Seasonal *M/D* of epilimnetic water of Green and Linge lakes and of a deepwater (43 m) and shallow-water (2 m) site on Trout Lake.

three lakes. Previously, it had been found that *M/D* is a good predictor of fish mercury concentrations (Winfrey and Rudd 1990; Hecky et al. 1991). The present study adds further evidence that the radiochemical methods (Furutani and Rudd 1980; Ramlal et al. 1986; Winfrey and Rudd 1990) do assay the relative methylating activity of the natural microbial populations. Even though the radiochemical methods are not quantitative, these data indicate that the radiochemical approach can also be successfully used (e.g., Xun et al. 1987; Kerry et al. 1991; Hecky et al. 1991; Miskimmin et al. 1992) to provide valuable insights into factors controlling natural methylating and demethylating populations and net methyl mercury production.

Sites of Methyl Mercury Production

Studies of microbial processes in lake sediments often concentrate only on deep hypolimnetic sediments to the exclusion of the shallower, epilimnetic sediments. Our data showed that epilimnetic methylation rates during summer were 20- to 40-fold higher than in hypolimnetic sediments (Fig. 3). During fall and winter, rates were low at both epilimnetic and hypolimnetic sites. The large epilimnetic to hypolimnetic differences during summer plus the fact that epilimnetic sediments often constitute most of the sediment surface of lakes lead us to conclude that most of the in-lake methylation in this type of oligotrophic lake occurs in the epilimnetic sediments during summer stratification.

Bloom and Watras (1989) observed elevated methyl mercury concentrations in the hypolimnetic water column of Little Rock Lake near the oxic–anoxic interface, suggesting that this might be a site of active methylation. We observed no such peak in the hypolimnion of Green Lake probably because the bottom waters of Green Lake, which is very oligotrophic, did not become anoxic during summer stratification. In our lakes, the oxic–anoxic interface was always in the sediments at 2–15 mm below the sediment–water interface (Sweerts 1990). Thus, our finding that epilimnetic sediments were more active in methylation than the hypolimnetic sediments does not exclude the possibility that anoxic processes are important methylators. For

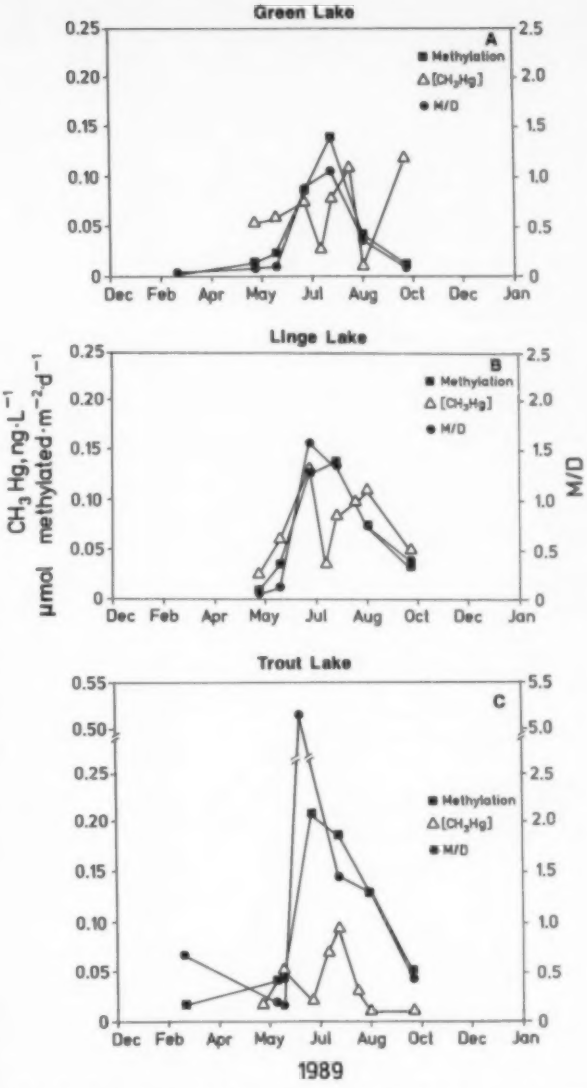

FIG. 7. Comparison of epilimnetic specific methylation, *M/D*, and water methyl mercury concentration in (A) Green lake, (B) Linge Lake, and (C) Trout Lake.

example, sulphate reducers may be important methylators (Gilmour and Henry 1991; Kerry et al. 1991) and they are active in epilimnetic sediments (Kelly and Rudd 1984).

Factors Controlling Methyl Mercury Production

The high epilimnetic methylation rates, as compared with hypolimnetic rates, were not related to sediment mercury concentrations, which showed no trend with lake depth (Table 2). One might conclude that the higher methylation rates occurred because of the higher respiration rates (i.e., higher rates of general microbial activity) in the epilimnetic sediments. However, the epilimnetic/hypolimnetic differences in methylation rates during summer were much larger (usually 20- to 40-fold) than

was the case for general sediment respiration (two- to fourfold, Fig. 2). This may indicate that mercury-methylating bacteria were particularly adapted to the epilimnetic environment and that a factor other than microbial activity was primarily responsible for the high epilimnetic methylation rates.

Data from several studies of our own and others suggest that the higher epilimnetic temperatures may be an important factor that stimulated epilimnetic methylation. For example, it has been found that methylation rates peak during midsummer (Korthals and Winfrey 1987; Ramsey and Ramlal 1987). We found that the water column methylation rates (data not shown) and *M*/*D*'s (Fig. 6) were higher in the smaller, warmer lakes than in the colder, larger lakes.

On the short term (weeks), we did not see a direct relationship between temperature and methylation rates. For example, in the spring of 1988, which was unusually warm, epilimnetic temperatures peaked in May as did sediment respiration rates (Fig. 1 and 2). At that time, methylation rates were very low, even though respiration rates were high. As in other studies (e.g., Korthals and Winfrey 1987), it appeared that an extended period of high temperature was required to stimulate the development of the mercury-methylating communities.

The combination of divergent patterns of methylating and demethylating activity with temperature was strongly expressed by the *M*/*D* ratios (Fig. 5) which were very high at warm temperatures in the epilimnetic sediments during midsummer, but decreased as the water cooled.

In contrast with methylation, demethylation appeared to be favoured by low temperature. During summer, demethylation rates were higher in cold hypolimnetic sediments than in warmer epilimnetic sediments (Fig. 4). There also appeared to be an overall negative relationship of sediment demethylation rates with temperature in the epilimnetic sediments, which is discussed in more detail in the companion paper (Bodaly et al. 1993). These results are similar to those of Korthals and Winfrey (1987) who found that peak demethylation activity occurred during late June. This was followed by decreasing activity over the summer with a steady rate through to December, gradually increasing until their last measurement in April. Similar results were also found during a study of mercury methylation and demethylation in reservoirs (Ramsey and Ramlal 1987), where maximum methylation rates occurred later in the season than did maximum demethylation rates.

The major conclusion of our study was the substantial contribution of the epilimnetic sediments to the microbial activity of the lakes. This was especially true for mercury-methylating activity and suggests that these important sites should not be ignored in a comprehensive study of mercury biogeochemistry.

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Mercury Concentrations in Fish Related to Size of Remote Canadian Shield Lakes[1]

R.A. Bodaly, J.W.M. Rudd, and R.J.P. Fudge

Department of Fisheries and Oceans, Central and Arctic Region, Freshwater Institute, 501 University Crescent, Winnipeg, Man., Canada R3T 2N6

and C.A. Kelly

Department of Microbiology, University of Manitoba, Winnipeg, Man., Canada R3T 2N2

Bodaly, R.A., J.W.M. Rudd, R.J.P. Fudge, and C.A. Kelly. 1993. Mercury concentrations in fish related to size of remote Canadian Shield lakes. Can. J. Fish. Aquat. Sci. 50: 980–987.

Mercury concentrations in planktivorous, omnivorous, and piscivorous fishes were inversely related to lake size in six lakes in northwestern Ontario. The lakes were remote from direct anthropogenic influences and ranged in surface area from 89 to 35 000 ha. Fish mercury concentrations were not related to ratios of drainage basin area to lake size, to ratios of epilimnetic area to lake size, to lake alkalinity, or to the concentration of mercury in lake sediments. Rates of mercury methylation (*M*) were positively dependent on water temperature whereas rates of methyl mercury demethylation (*D*) were inversely related to temperature. Thus, *M*/*D* was strongly temperature dependent. Mercury concentrations in four fish species were significantly positively correlated with mean epilimnetic water temperatures (r^2's ranged from 0.66 to 0.88). This suggested that higher water temperatures in smaller lakes during the open-water season influenced *M*/*D* ratios and were the cause of higher fish mercury levels. No lake size related variation was observed in the mercury concentrations in benthivorous fishes. Our hypothesis that epilimnetic temperature affects mercury concentrations in fish deserves further attention, given the possible effects of climate warming on mercury contamination of fishery resources.

Les concentrations de mercure chez les poissons planctivores, omnivores et piscivores étaient inversement proportionnelles à la taille du plan d'eau dans six lacs du nord-ouest de l'Ontario. Les lacs étaient isolés des influences humaines directes, et leur superficie variait de 89 à 35 000 ha. Les concentrations de mercure dans le poisson n'étaient pas liées au rapport de la superficie du bassin versant à la taille du lac, au rapport de la superficie de l'épilimnion à la taille du lac, à l'alcalinité du lac ni à la concentration de mercure dans les sédiments. Les taux de méthylation du mercure (*M*) étaient positivement dépendants de la température de l'eau, tandis que les taux de déméthylation du méthylmercure (*D*) étaient inversement liés à la température. Le rapport *M*/*D* était donc fortement dépendant de la température. Les concentrations de mercure chez quatre espèces de poissons présentaient une corrélation positive significative avec la température moyenne de l'eau dans l'épilimnion (r^2 entre 0,66 et 0,88). Cela semble indiquer que l'élévation de la température de l'eau dans les petits lacs pendant la saison d'eau libre influait sur le rapport *M*/*D* et était à l'origine des concentrations plus fortes de mercure chez les poissons. On n'a observé aucune variation liée à la taille du lac dans les concentrations de mercure chez les poissons qui se nourrissaient sur le fond. Notre hypothèse, selon laquelle la température de l'épilimnion aurait un effet sur les concentrations de mercure chez les poissons, doit être examinée de plus près, étant donné les effets que pourrait avoir le réchauffement climatique sur la contamination par le mercure des ressources halieutiques.

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The bioaccumulation of mercury in fish is controlled by several interrelated factors. These include fish trophic status, physiology, and age, as well as the microbial production, concentration, and bioavailability of methyl mercury in the ecosystem. When environmental conditions are perturbed, one or more of these factors are often affected, and this can result in elevated concentrations of methyl mercury in fish. For example, point-source additions of inorganic mercury increase the concentration of mercury in fish (Armstrong and Scott 1979). Lake acidification increases both methyl mercury production (Xun et al. 1987) and fish mercury concentrations (Wiener et al. 1990) as does the construction of hydroelectric reservoirs where methylation rates are stimulated by the decomposition of flooded organic material (Bodaly et al. 1984; Hecky et al. 1991).

Fish mercury concentrations are also high (i.e., 0.5–1.0 ppm or greater) in some remote lakes that have no known history of human disturbance or mercury point-source inputs (e.g., OME 1990), suggesting that either the production or bioaccumulation of methyl mercury is elevated in these lakes. Our understanding of the biogeochemistry of mercury in aquatic environments would be significantly advanced if the reason(s) for these high mercury concentrations of fish under seemingly pristine conditions were known.

[1]This is one of a series of NOLSS papers dealing with the lake size series of northwestern Ontario.

TABLE 1. Physical and limnological characterization of the six study lakes in northwestern Ontario. Data from Fee et al. (1989) unless otherwise noted.

	Green	Orange	Linge	Musclow	Sydney	Trout
Lake area (ha)	89	167	706	2219	5748	34690
Mean depth (m)	7.7	14.4	8.4	19.3	20.0	13.7
Lake volume ($m^3 \times 10^5$)	68.5	240.5	591.0	4282.7	11496.0	47585.3
Area of drainage basin (ha)	323	1270	3687	35067	55297	106533
Ratio of drainage basin area to lake area	3.63	7.60	5.22	15.80	9.62	3.07
Ratio of drainage basin area to lake volume	4.71	5.28	6.22	8.19	4.81	2.24
Ratio of direct drainage area (including lake) to lake volume (E.J. Fee, unpublished)	0.474	0.529	0.624	0.821	0.454	0.226
Ratio of epilimnetic sediment surface area to epilimnetic volume (Fee and Hecky 1992)	0.08	0.04	0.09	0.02	0.04	0.04
Mean (June–Aug.) epilimnetic water temperature (1986–89) (E.J. Fee, unpublished)	20.04	20.13	19.49	18.64	18.58	16.88
Specific conductance (at 25°C) ($\mu S \cdot cm^{-1}$)	28	48	30	43	41	62
pH	7.21	7.50	7.30	7.53	7.47	7.69
DOC ($\mu mol \cdot L^{-1}$)	590	740	690	830	600	410
Alkalinity ($\mu eq \cdot L^{-1}$)	177	373	209	337	292	545
Ca ($\mu mol \cdot L^{-1}$)	69	132	87	126	107	212
Total Hg in sediments (0–2 cm) < 20 m ($\mu mol \cdot m^{-2}$) (Ramlal et al. 1993)	0.32	—	0.45	—	0.25	0.22
Ratio of epilimnion surface area to lake volume	0.41	0.18	0.59	0.11	0.16	0.36

We studied mercury biogeochemistry in a size series of six remote Canadian shield lakes in northwestern Ontario by relating the mercury concentrations in fish to specific rates of mercury methylation and demethylation. Our null hypothesis was that mercury concentrations in fish would be unrelated to lake size because of similar mercury loadings on a lake area or lake volume basis and because of similar internal lake processes and water chemistry, e.g., primary productivity, dissolved organic carbon (DOC), and pH, which were related to mercury in fish in other studies (e.g., Håkanson et al. 1988).

Study Lakes

The Northwest Ontario Lake Size Series (NOLSS) lakes are in a remote region of northwestern Ontario, Canada (51°N, 94°W) (Fee and Hecky 1992). The six lakes are located in the boreal forest in an area underlain by Canadian Shield bedrock. Climate is severe continental with cold winters and warm summers. The lakes are generally oligomesotrophic with specific conductances ranging from 28 to 62 $\mu S \cdot cm^{-1}$ (Table 1). The major variable in this lake series was surface area, which ranged from 89 to 35 000 ha. The lakes were chosen so that other key physical variables, including mean depths, the ratio of watershed area to lake size, and water residence times, were as similar as possible (Table 1).

Chemical parameters (pH, DOC, nutrient content, primary productivity) also varied little among the lakes (Table 1). Because they were located in the same geological setting within a 50-km radius, they likely had similar inputs of mercury per unit area from the watersheds and airsheds. The fish communities of these lakes were typical of those in cool- and cold-water oligo- and mesotrophic lakes of the region, being composed of a relatively small number of large fish species, such as walleye (*Stizostedion vitreum*), northern pike (*Esox lucius*), white sucker (*Catostomus commersoni*), cisco (*Coregonus artedi*), lake whitefish (*Coregonus clupeaformis*), and yellow perch (*Perca flavescens*).

Methods and Materials

Sampling of Fish

Fish were captured from all six study lakes in the open-water seasons of 1987, 1988, and 1989. Gill nets of mesh sizes varying from 16 to 133 mm stretch measure were used. Fork length (± 1 mm) was determined for all fish. Fish were sampled for ageing structures as follows; walleye, operculum; northern pike, cleithrum; white sucker and cisco, pectoral fin; yellow perch, dorsal spine; we also aged yellow perch by analysis of the length frequency distributions. Opercula and cleithra were cleaned, dried, and aged with a dissecting microscope. Pectoral fins and dorsal spines were cut to size, dried, embedded in epoxy, thin-sectioned with an Isomet low-speed saw, and mounted on glass slides with Diatex medium. The sections were aged with a compound microscope (projected image).

A sample of white muscle (>25 g) was removed from the upper left side below the dorsal fin of all fish. Samples were frozen soon after fish capture. Total mercury concentrations were determined by the method of Hendzel and Jamieson (1976). Determinations of National Research Council of Canada

standard reference materials (DORM-1) were made in the course of analyses of fish tissues from the NOLSS lakes. The certified value of the reference material was 0.798 ± 0.074 $\mu g \cdot g^{-1}$ and the mean of 17 determinations of this material was 0.805 ± 0.057 $\mu g \cdot g^{-1}$.

When there was a significant linear relationship between log(mercury concentration) and log(fish length), the mean mercury concentration for that sample was adjusted to a standardized fish length. The following standardized fork lengths were chosen to be within the range of fork lengths in all samples analysed: walleye, 400 mm; northern pike, 600 mm; white sucker, 350 mm; cisco, 180 mm; yellow perch, 80 mm. When there was no significant relationshp between log(mercury concentration) and log(fish length) (three of the yellow perch samples, three of the cisco samples, and all of the lake whitefish samples), means unadjusted for fish size were used. Mean sizes for these samples were similar and size ranges were broadly overlapping. Mean mercury concentrations were also adjusted to the grand mean age for each species by linear regression. Mean mercury concentrations varied little from year to year, with no consistent patterns within or among species, and were therefore combined for all years.

Measurement of Specific Rates of Mercury Methylation and Demethylation

Specific rates of mercury methylation (M) and demethylation (D) and the ratio of methylation to demethylation (M/D) were estimated as described in Furutani and Rudd (1980) and Ramlal et al. (1986). Briefly, three epilimnetic cores were taken by a scuba diver. The top 2 cm of the cores was pooled and then split so that methylation or demethylation measurements could be done on similar sediments. Methylation was assayed after adding $^{203}HgCl_2$. Demethylation was assayed after adding $^{14}CH_3HgI$ and incubating the sediments at in situ temperature. Details of the coring procedures and the procedures used for assaying methylation and demethylation for this particular study are given in Ramlal et al. (1993). Four of the six lakes in the size series were sampled (Green, Linge, Sydney, and Trout). Methylation analyses were done during the summers of 1988 and 1989 and on one occasion during winter (February 1989). Demethylation was assayed only during the summer and winter of 1989.

Statistical Analyses

Simple correlation coefficients were calculated between mercury concentrations in fish and the following factors (see Table 1 for details): lake area, ratio of total drainage basin area to lake volume, ratio of direct drainage basin area (not through upstream lakes) to lake volume, ratio of epilimnion area to epilimnion volume, ratio of epilimnion area to lake volume, epilimnetic water temperature (June–August 1986–89), alkalinity, and DOC.

Alkalinity was highly correlated with pH, conductivity, and calcium concentrations and was used as a surrogate of those three variables. Numerous previous studies have demonstrated relationships between mercury concentrations in fish and pH (Håkanson et al. 1988; McMurtry et al. 1989; Cope et al. 1990; Grieb et al. 1990; Suns and Hitchin 1990; Wiener et al. 1990). However, none of the NOLSS lakes are acidic and the range of alkalinities was small. Ratios of drainage basin area and direct drainage area to lake volume were used to represent proportional rates of mercury loadings from the atmosphere via the watershed, on a total watershed basis, and as uninfluenced by upstream lakes. Ratios of epilimnetic area to epilimnetic and lake volumes were included because most mercury methylation activity occurs in the epilimnion of these lakes (Ramlal et al. 1993). DOC has been related to mercury concentrations in fish in a number of studies (Verta et al. 1986; Grieb et al. 1990).

Results

Mercury Concentrations in Fish

Mercury concentrations in northern pike and walleye (both piscivores), cisco (a planktivore), and yellow perch (an omnivore) varied greatly (three- to fourfold) among lakes (Fig. 1). Mean mercury concentrations in these species were inversely related to lake size (Fig. 1). In general, size-adjusted mercury concentrations in northern pike, walleye, cisco, and yellow perch in the various lakes followed the pattern Green ≈ Orange > Linge > Musclow ≈ Sydney > Trout. In white sucker and lake whitefish (both benthivores), there was relatively little variation in mean mercury concentrations among lakes, and variation present seemed unrelated to lake size (Fig. 2).

Data adjusted by age was less extensive than those adjusted for length because some fish were not aged; however, similar patterns were evident for the age-adjusted means as for the length-adjusted means (Fig. 3).

Our most uniform data set for fish mercury was a subset from analysis of yellow perch sampled in the same year from all six lakes (Fig. 4). All of these yellow perch samples were taken within a 19-d period. All fish were the same age (1+), and mean size varied little among lakes, from 70 to 81 mm. Mean mercury concentrations in these samples ranged from 0.04 to 0.14 $\mu g \cdot g^{-1}$ and were highest in Green Lake, lowest in Trout Lake, and inversely related to lake size (Fig. 4). Thus when the most uniform data set was considered, the relationship of lake size to fish mercury concentrations was clearly evident.

Mercury Methylation, Demethylation, and Epilimnetic Temperatures

Average epilimnetic temperatures in June–August ranged from <17 to >20°C and were higher in the smaller lakes (Table 1). The epilimnia of the smaller lakes warmed more quickly in the early summer whereas temperatures were similar in late summer and autumn (Fig. 5).

To examine the relationship between temperature and rates of methylation, demethylation, and M/D, we plotted all the data from epilimnetic sites for the four lakes that were sampled (Fig. 6). Temperature was measured at the sediment–water interface at each sampling site at the time of sampling, and this was the incubation temperature for the sediment samples. We only included data from epilimnetic sites in this analysis because >90% of the methylating activity in these lakes occurred in the epilimnion (Ramlal et al. 1993).

There was a general positive relationship between temperature and methylation rate (Fig. 6A). High methylation rates (i.e., >0.12 $\mu mol \cdot m^{-2} \cdot d^{-1}$) occurred only in July and August at water temperatures >16°C. Methylation rates at temperatures >16°C during June were low; these low early summer rates at high temperatures have been observed elsewhere (Hecky et al. 1991) and indicate that there is a lag period before seasonal methylating activity begins. Methylation rates were generally slow at cold temperatures (i.e., <9°C) during winter and spring. Except for the midwinter (February) demethylation

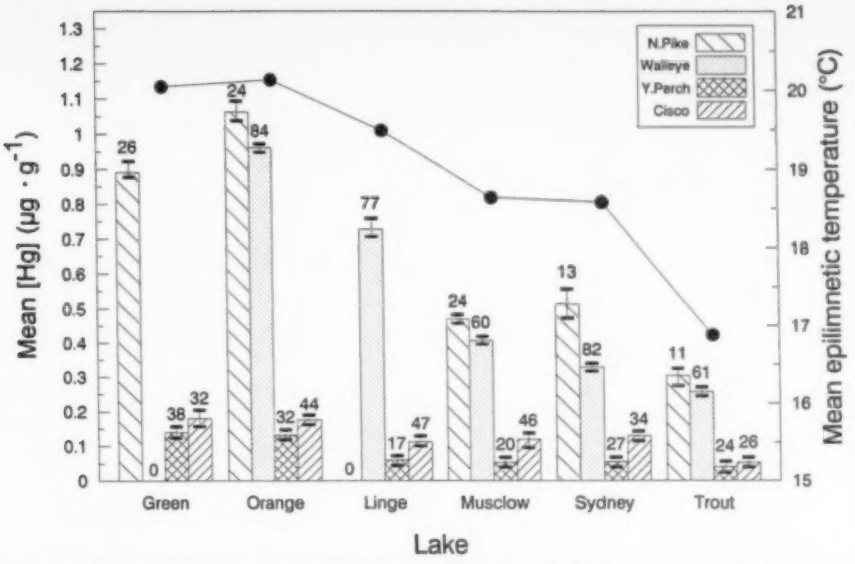

FIG. 1. Mean mercury concentrations in the axial muscle of northern pike, walleye, yellow perch, and cisco in the six study lakes. Most means are adjusted for fish size (see Methods and Materials for explanation). Lakes are arranged in order of smallest (Green) to largest (Trout) (see Table 1). Total sample sizes for each species from each lake (samples from 1987, 1988, and 1989 combined) are given above each bar. Error bars are 95% confidence intervals. Average epilimnetic water temperatures (Table 1) are superimposed.

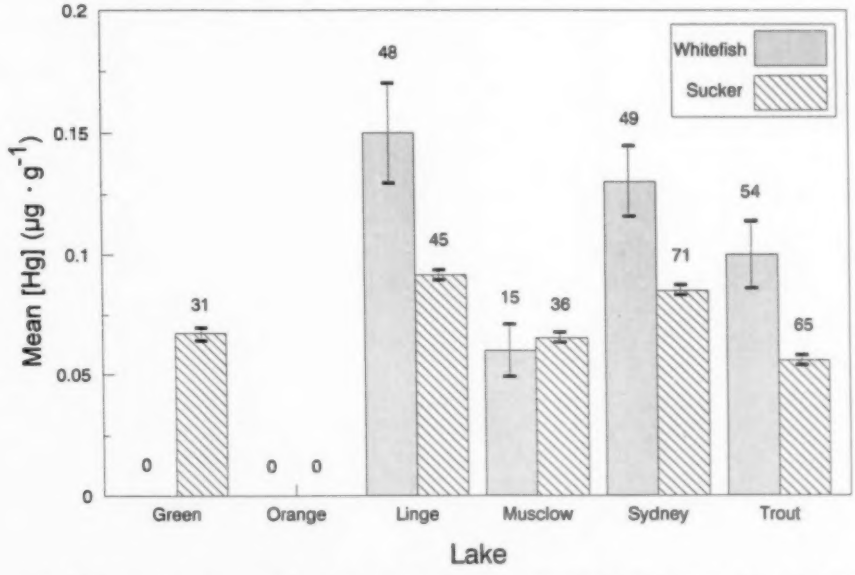

FIG. 2. Mean mercury concentrations in the axial muscle of lake whitefish and white sucker in the six study lakes. White sucker means are adjusted for fish size and lake whitefish means are unadjusted (see Methods and Materials for explanation). Lakes are arranged in order of smallest (Green) to largest (Trout). Total sample sizes for each species from each lake (samples from 1987, 1988, and 1989 combined) are given above each bar. Error bars are 95% confidence intervals.

measurements, which were very slow, there was a general negative relationship between specific demethylation rates and temperature (Fig. 6B). Demethylation rates were lowest in July and August, when methylating activity was greatest. As a result of the opposite relationship of methylation and demethylation with temperature, the *M*/*D* ratio had a strong positive relationship with temperature (Fig. 6C). *M*/*D* peaked during July when epilimnetic temperatures were at their maxima.

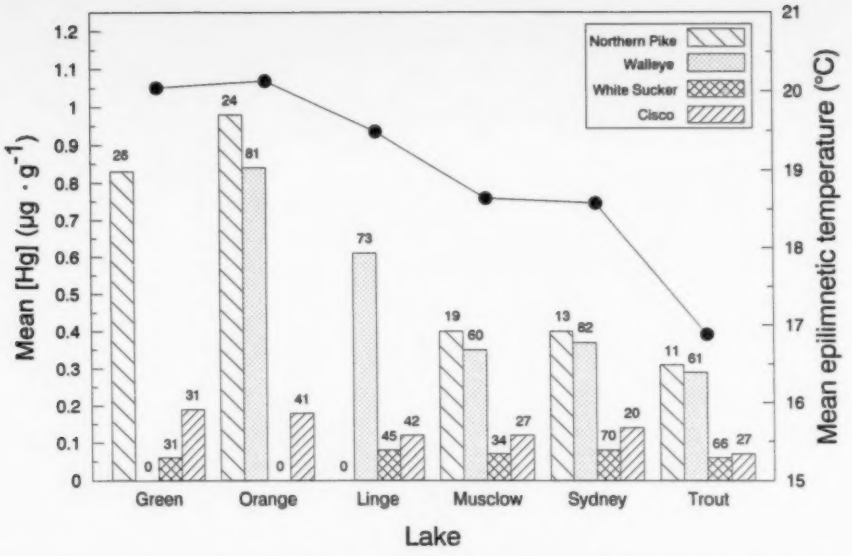

FIG. 3. Mean mercury concentrations in the axial muscle of northern pike, walleye, white sucker, and cisco in the six study lakes adjusted for fish age. Total sample sizes for each species from each lake (samples from 1987, 1988, and 1989 combined) are given above each bar.

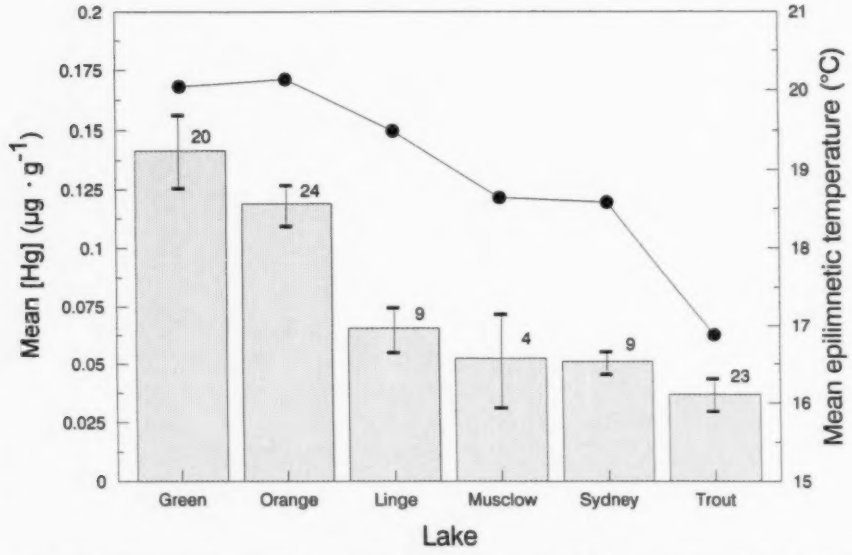

FIG. 4. Mean mercury concentrations in the axial muscle of yearling yellow perch in the six study lakes. Lakes are arranged in order of smallest (Green) to largest (Trout). All fish were sampled in 1989. Sample sizes are given above each bar. Error bars are 95% confidence intervals.

Relationships between Mercury Concentrations in Fish and Lake Characteristics

There were statistically significant linear relationships between mercury concentrations in northern pike, walleye, cisco, and yellow perch (Fig. 1) and some physical characteristics of the study lakes (Table 1). Epilimnetic temperature (positive slope) always showed the strongest relationship with mercury concentrations in these species (Table 2). Basin area, lake volume, and lake area also showed significant correlations with mercury in some of the species; however, the relationship of these variables with mercury concentration in fish was not surprising because lake temperature was related to lake size and basin area (Table 1; Fig. 5). No chemical parameters were significantly correlated with mercury in fish. A sequential Bonferroni adjustment of probability levels designed to take into account the number of correlation coefficients being examined (Rice 1989) indicated that only the correlation between cisco mercury and epilimnetic temperature was significant (adjusted

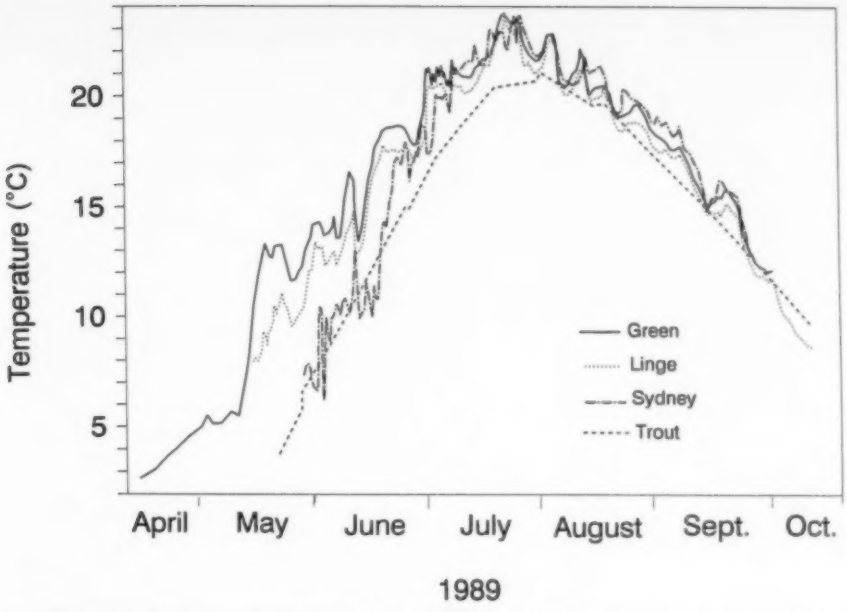

FIG. 5. Epilimnetic water temperatures of four NOLSS lakes, 1989. Temperatures were measured at 1 m depth.

TABLE 2. Characteristics of the study lakes which showed significant correlations with mercury concentrations in six fish species. Lake characteristics taken from Table 1. $^*P < 0.05$.

Species	Lake variable	r
Northern pike	Epilimnion temperature	0.94*
(n = 5 lakes)	Basin area	−0.89*
Walleye (n = 5 lakes)	Epilimnion temperature	0.89*
	Basin area	−0.86*
Cisco (n = 6 lakes)	Epilimnion temperature	0.92*
	Basin area	−0.84*
	Lake volume	−0.83*
	Lake area	−0.83*
Yellow perch (1989)	Epilimnion temperature	0.83*
(n = 6 lakes)	Basin area	−0.75*
Yellow perch (all years)	Epilimnion temperature	0.81*
(n = 6 lakes)		
White sucker	No significant correlations	
(n = 5 lakes)		
Lake whitefish	No significant correlations	
(n = 4 lakes)		

p required for $\alpha = 0.05$ was 0.0063). This adjustment is an extremely restrictive test of significance. No lake characteristics showed significant correlations with mercury concentrations in white sucker or lake whitefish (Table 2).

Discussion

Our results indicate a strong inverse relationship between lake size and mercury concentration in piscivorous, planktivorous, and omnivorous fishes. Lake size – fish mercury relationships of the magnitude reported here have not been noted previously in the literature. Håkanson et al. (1988) observed a weak but significant inverse correlation between lake size and mercury concentrations in northern pike in Swedish lakes, but did not discuss possible causes. McMurtry et al. (1989) found, for Ontario lakes, that mercury in lake trout (*Salvelinus namaycush*) and smallmouth bass (*Micropterus dolomieu*, formerly *M. dolomieui*) was *positively* related to lake area (and watershed area); however, these variables explained a relatively small proportion of the variation in fish mercury concentrations in their study. Previous studies have not examined such a carefully selected series of lakes as the present study, in which key variables (e.g., climate, watershed ratios, mean depth, and chemical and biological characteristics) have been similar, with only lake size varying appreciably among lakes. This may explain the lack of previous reports of strong lake size – fish mercury level relationships.

The lake size – fish mercury relationship observed in this study did not seem to be caused by variation in the chemical characteristics of the lakes. Recent studies have demonstrated that lakes with low pH and low buffering capacity typically have fish with high mercury concentrations (Håkanson et al. 1988; McMurtry et al. 1989; Cope et al. 1990; Grieb et al. 1990; Suns and Hitchin 1990; Wiener et al. 1990). Water hardness (calcium concentrations) has also been implicated as affecting mercury uptake by fish (McFarlane and Franzin 1980; Rodgers and Beamish 1983). Alkalinity, calcium concentration, pH, and conductance are strongly interrelated in the NOLSS lakes (Table 1). Although all four of these chemical variables were lowest in the smallest lake and highest in the largest lake, they did not vary in relation to lake size among the middle four lakes and were not correlated with fish mercury concentrations. In addition, the measured rates of mercury methylation and methyl mercury demethylation and the *M/D* ratios were unrelated to water alkalinity over the narrow range tested in our study (J.W.M. Rudd, unpublished data).

Much evidence points to the importance of atmospheric mercury deposition, either directly to lakes or via their watersheds, in determining fish mercury levels. Estimated direct annual

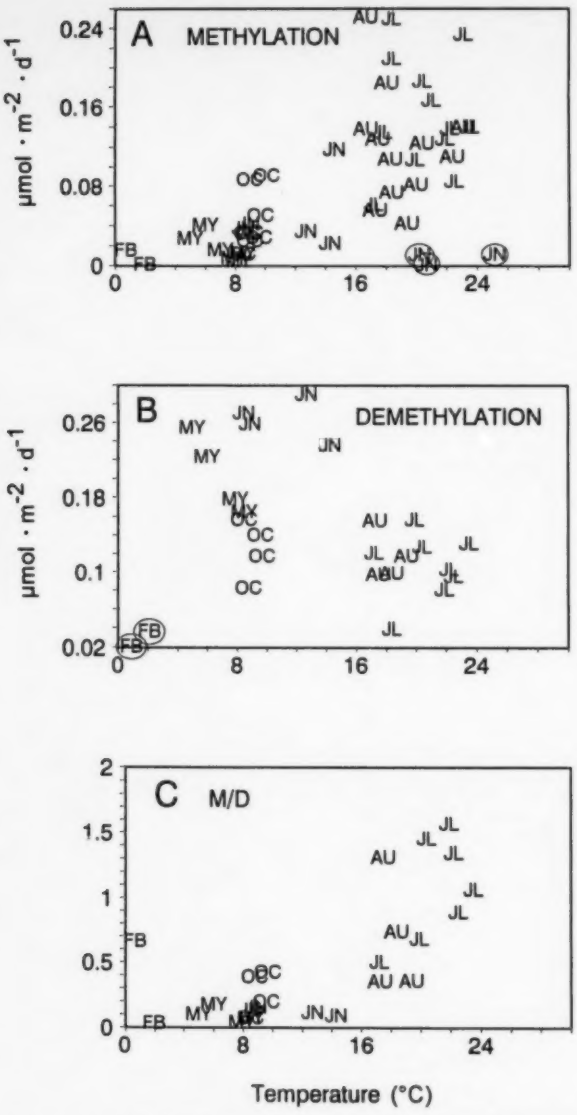

FIG. 6. Rates of (A) mercury methylation and (B) methyl mercury demethylation and (C) *M/D* ratios versus temperature, 1988–89, for Green, Linge, Sydney, and Trout lakes. Each data point is an individual determination with the month of measurement given as follows: FB = February; MY = May; JN = June; JL = July; AU = August; OC = October. Circled (June) data points in Fig. 6A indicate anomolously low methylation rates during early summer; circled (February) data points in Fig. 6B indicate low demethylation rates during winter. See Results section for full explanation.

inputs of mercury from the atmosphere to lakes are probably quite large relative to the amount of mercury in the fish community (Fitzgerald and Watras 1989; Wiener et al. 1990; Fitzgerald et al. 1991). Watershed sources of mercury may also influence fish mercury levels (Lee and Hultberg 1990; Mierle 1990). A number of relationships between fish mercury concentrations and watershed area have been noted (Verta et al. 1986; McMurtry et al. 1989; Suns and Hitchin 1990), and watershed characteristics may affect mercury yield (Verta et al. 1986; Aastrup et al. 1991). Mercury concentrations in fish in a set of Ontario lakes were related to mercury loadings (Johnson 1987). However, loadings of mercury, either directly from the atmosphere or indirectly from the watershed, were probably similar per unit of lake area for the lakes studied here. Neither ratios of total drainage basin area nor direct drainage area to lake size were significantly correlated with mercury concentrations in fish in our study lakes. Ramlal et al. (1993) concluded that most of the methyl mercury in solution in the NOLSS lake waters was produced within the lakes, and our conclusions regarding the relative unimportance of watershed size and characteristics support their finding.

Mercury concentrations in fish in lakes have been related to sediment mercury concentrations in some studies (Håkanson et al. 1988; Cope et al. 1990). However, variation in sediment mercury concentrations cannot explain the differences in fish mercury observed in this study. First, there was little variation in mercury concentrations in surficial sediments among the NOLSS lakes (concentrations of total mercury ranged from 0.22 to 0.45 $\mu mol \cdot m^{-2}$), and this is probably further indication that mercury loading rates do not vary significantly among the NOLSS lakes. Variation in sediment mercury concentrations was not related to lake size because Linge Lake had the highest sediment mercury concentration (Table 1) but contained fish with intermediate mercury concentrations.

Mercury concentrations in fish in the NOLSS lakes were strongly related to epilimnetic temperatures. The smaller lakes tended to warm earlier in the spring and early summer and had higher midsummer temperatures, resulting in higher average epilimnetic temperatures (Fig. 5; Table 1). From 66 to 88% of the variation in size-adjusted fish mercury concentrations (Fig. 1) was explained by mean epilimnetic water temperature (Table 2). The linkage of fish mercury to water temperature may result from the influence of temperature on rates of mercury methylation and demethylation (Fig. 5; Wright and Hamilton 1982; Callister and Winfrey 1986). Both atmospheric and aquatic loadings of inorganic mercury greatly exceed the amount of mercury in the fish community (Fitzgerald and Watras 1989; Mierle 1990; Wiener et al. 1990), and the pool of inorganic mercury in lake sediments vastly exceeds the methylated pool in the fish (Fitzgerald and Watras 1989). Thus, rates of mercury methylation and demethylation are the probable factors controlling fish mercury concentrations in lakes, as in reservoirs (Hecky et al. 1991).

Most methylation appears to be taking place in the epilimnion of our study lakes (Ramlal et al. 1993). The hypothesis that fish mercury levels are strongly influenced by epilimnetic water temperatures via their control on *M/D* ratios is further supported by the observations of Ramlal et al. (1993); they showed that methyl mercury concentrations in water tend to follow estimates of *M/D*, even though actual concentrations did not seem to be strongly related to lake size.

In addition to influencing *M/D* ratios, the higher water temperature in small versus larger lakes could further enhance mercury bioaccumulation due to the effect of temperature on fish metabolic rates. An increase in water temperatures will increase metabolic rates in fish. Both feeding rates and gill ventillation rates will tend to be higher at higher temperatures, leading to higher rates of methyl mercury uptake from food and water.

The reason for the lack of a lake size related trend in mercury concentrations in benthivorous fishes is unclear. Benthivorous fishes such as white sucker and lake whitefish may utilize epi-

limnetic habitat to a lesser degree than the piscivorous and planktivorous fishes sampled in this study. If so, benthivores would be less exposed to epilimnetic waters and food sources with higher methyl mercury concentrations. Inhabiting cooler water, benthivores may have lower average metabolic rates and slower rates of food uptake and gill ventillation and slower rates of uptake of methyl mercury.

The hypothesis that lake size and epilimnetic water temperatures may be variables influencing fish mercury concentrations may explain anomolously high fish mercury concentrations in some remote lakes. This hypothesis could be tested by examining epilimnetic water temperature and fish mercury data sets for larger numbers of lakes.

Temperature should be considered an important variable influencing mercury levels in fish along with previously determined variables, such as loading rates, watershed ratios, and pH. Our findings have direct implications for the potential effects of long-term climate warming on fish mercury concentrations. Schindler et al. (1990) noted that an increase in average air temperatures, coupled with more frequent forest fires, has increased lake temperatures, the length of the ice-free period, and thermocline depths in a boreal lake. Such changes, which have the potential to increase overall lake *M/D* ratios and fish mercury levels, will not be remedied by regional action by regulatory agencies.

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Proton Transport and Chloride Cells in the Gill of Rainbow Trout (*Oncorhynchus mykiss*)

Nils Petter Berg Justesen

Institute of Anatomy, University of Bergen, Årstadveien 19, N-5009 Bergen, Norway

Torbjørn Dall-Larsen

Department of Biochemistry, University of Bergen, Årstadveien 19, N-5009 Bergen, Norway

and Leiv Klungsøyr

Stabekk College, Ringstadbekkveien 105, N-1340 Bekkestua, Norway

Justesen, N.P.B., T. Dall-Larsen, and L. Klungsøyr. 1993. Proton transport and chloride cells in the gill of rainbow trout (*Oncorhynchus mykiss*). Can. J. Fish. Aquat. Sci. 50: 988–995.

Particulate preparations from freshwater rainbow trout (*Oncorhynchus mykiss*) gill homogenates contain an active magnesium ion activated ATPase that transports protons into the vesicles. Oligomycin at a concentration of 20 $\mu g \cdot mL^{-1}$ had little effect on the proton transport, which was completely inhibited by *N*-ethylmaleimide. This inhibition was partly counteracted by dithiothreitol. Proton transport in freshwater trout gill submitochondrial particles was completely inhibited by oligomycin. When freshwater trout were kept for 40 min in water equilibrated with air containing 5% carbon dioxide, their gill lamellar epithelium contained many cells densely covered with erect microvilli. After changing to water bubbled with room air, the cells rapidly lost their microvilli. Control fish that were killed immediately after removal from the storage tank also had microvilli, but less erect and fewer in number. Labelled latex microspheres and dextran were used as markers for external medium uptake into gill epithelial cells. The fish were pretreated by bubbling the water by air containing 5% carbon dioxide, and the uptake took place when the water was bubbled with room air.

Des préparations particulaires obtenues à partir d'homogénats de branchies de truite arc-en-ciel (*Oncorhynchus mykiss*) d'eau douce contiennent une ATPase activée par l'ion magnésium et qui sert à transporter des protons dans les vésicules. L'oligomycine à la concentration de 20 $\mu g \cdot mL^{-1}$ n'a pas eu d'effet important sur le transport de protons qui était complètement inhibé parle *N*-éthylmaléimide. Cette inhibition était partiellement bloquée par le dithiothreitol. Le transport de protons dans les particules inframitochondriales des branchies de truite d'eau douce était complètement inhibé par l'oligomycine. Lorsque les truites d'eau douce étaient gardées pendant 40 min dans de l'eau en équilibre avec de l'air contenant 5% de dioxyde de carbone, l'épithélium lamellaire de leurs branchies contenait de nombreuses cellules couvertes de microvillosités dressées et très denses. Après barbotage avec de l'air de la pièce, les cellules ont perdu rapidement leurs microvillosités. Les poissons témoins sacrifiés immédiatement après la sortie du réservoir avaient également des microvillosités, mais ces dernières étaient moins dressées et leur nombre était plus petit. Des microsphères de latex marquées et du dextran ont été utilisés comme marqueurs de l'absorption depuis le milieu extérieur dans les cellules épithéliales de branchie. Les poissons ont fait l'objet d'un prétraitement par barbotage de l'eau avec de l'air contenant 5 % de dioxyde de carbone et l'absorption a eu lieu lorsque le barbotage se faisait avec de l'air de la pièce.

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In teleost fish, which are mostly carnivorous, amino acids constitute a major energy source. Amino acids are broken down to carbon dioxide and nearly equimolar quantities of bicarbonate and ammonium ions (Atkinson and Camien 1982). The acidic carbon dioxide is eliminated through the gill epithelium.

Mammals remove bicarbonate base by way of urea synthesis in the liver, which prevents the development of alkalosis (Atkinson and Bourke 1984). In teleost fish, some bicarbonate may be eliminated through exchange with chloride ions (De Renzis and Maetz 1973; Heisler 1984); however, most is probably eliminated by the passive diffusion of ammonia, down a concentration gradient that is created and sustained by the rapid perfusion of water over the gill epithelium.

It is conceivable that under standard conditions, the combination of bicarbonate and ammonia base elimination may represent some overexcretion of base in teleosts (Klungsøyr 1987). This would require proton excretion, possibly in excess of what is exchanged for sodium ions. When the fish is exposed to acid water, or when it produces extra metabolic acid, the pumping of protons may have to be increased much above normal.

Finally, a flexible capacity for gill proton pumping is probably necessary for regulatory purposes. We observed (Klungsøyr 1987) that the gills of freshwater rainbow trout (*Oncorhynchus mykiss*, formerly, *Salmo gairdneri*) contain a magnesium ion dependent ATPase, which may be prepared in closed vesicles, of partly everted orientation. These vesicles are capable of an inward transport of protons during hydrolysis of

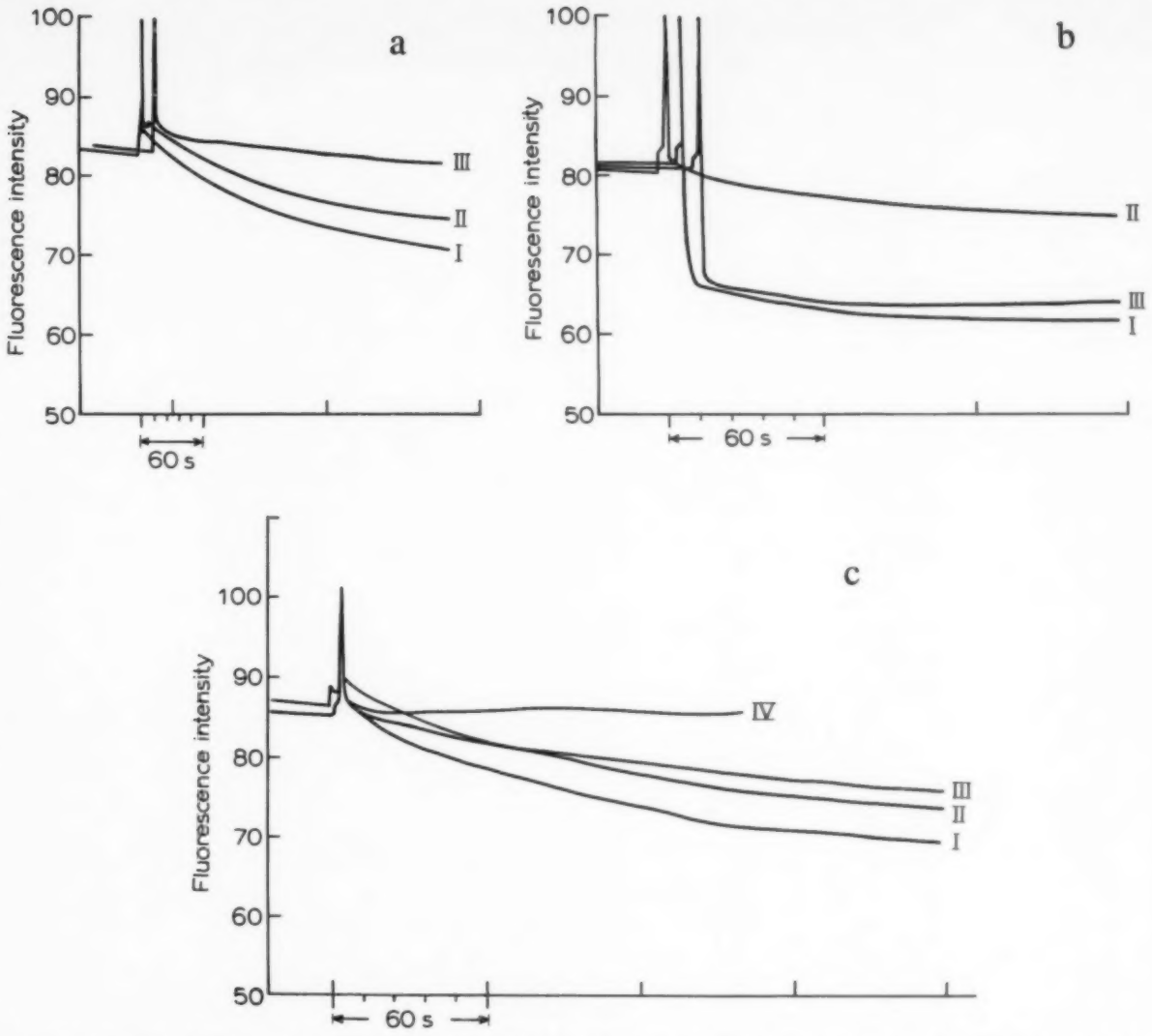

FIG. 1. Representative experiments with proton uptake into (a and c) gill epithelial vesicles and (b) submitochondrial particles prepared from gill epithelium. Uptake is registered as a decrease in acridine orange fluorescence; for details, see Materials and Methods. Trace I, control; trace II, reaction mixture containing 20 μg oligomycin·mL^{-1}; trace III, vesicle preparation containing 1 mM DTT in Fig. 1b and 1c preincubated with 1 mM NEM for 7 min; trace IV, vesicle suspension preincubated for 7 min with 3 mM NEM.

ATP. This proton transport was partly insensitive to oligomycin. An electrogen proton pumping ATPase located in fish gill has recently been described (Randall and Lin 1991).

Increasing epithelial cell apical membrane area, probably at least partly because of ATPase insertion, has been reported by several authors (Madsen and Tisher 1983; Stetson and Steinmetz 1983; Dixon et al. 1986; Brown et al. 1987). This work had three objectives: (1) We studied the structure of rainbow trout gill epithelial surface in fish that had been treated with carbon dioxide to look for evidence of membrane extension and in fish that had been returned from carbon dioxide enriched water to neutral water. (2) We looked for evidence of endocytosis during the period of recovery from carbon dioxide treatment in neutral water. (3) The biochemical studies (Klungsøyr 1987) on proton transport in vesicles were extended in order to test for sensitivity to inhibitors other than oligomycin and to compare the vesicular membrane preparation with gill submitochondrial particles. In fish gill, pump insertion might be maximal under hypercapnic conditions, and we wanted to search for evidence of increases in membrane area or chloride cell number. Conditions with low carbon dioxide would provide controls with expected evidence of a smaller membrane area. We have used the expression "chloride cells" synonymous with the mitochondria-rich cells described as the probable site of the proton-pumping ATPase (Schwartz and Al-Awqati 1986).

Materials and Methods

Rainbow trout adapted to freshwater were obtained from the Fisheries Research Institute, Department of Aquaculture

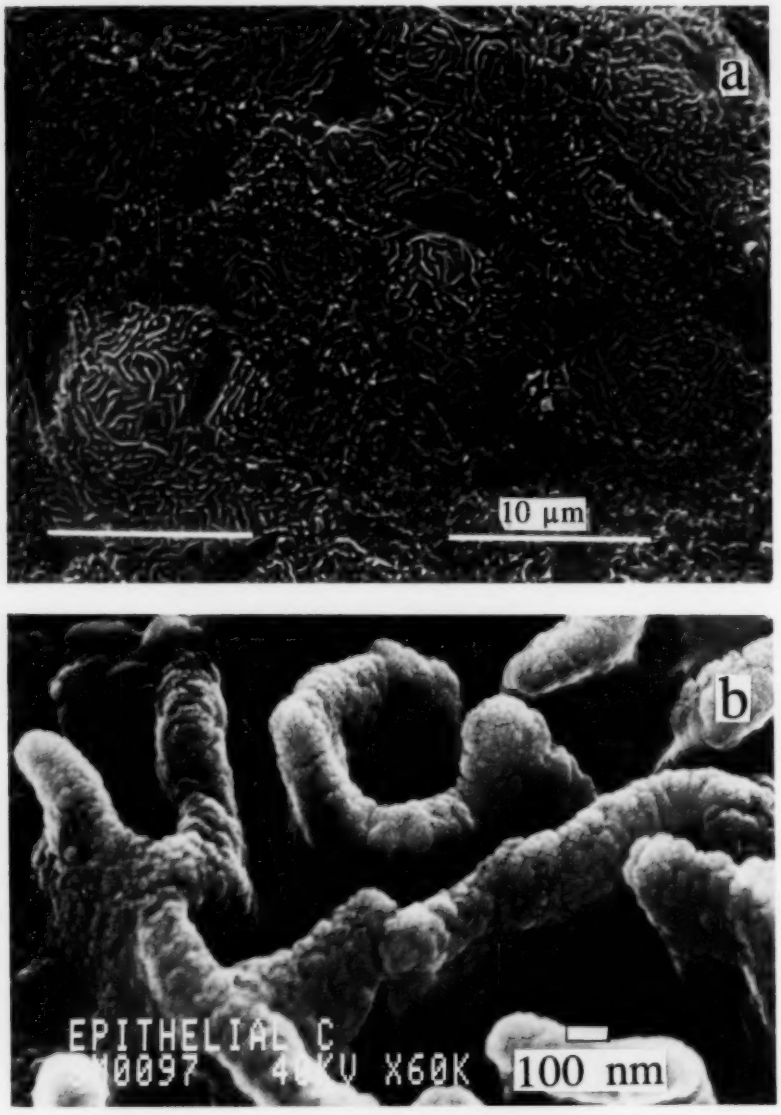

FIG. 2. (a) Microridges on gill epithelial cells. Note "windows" in epithelial cell layer, probably containing emerging chloride cells. Magnification 3200 ×. (b) Microridges at 60 000 ×.

(Matredal Division), or from local fish farmers. Net weights of fish were between 50 and 200 g.

For preparation of vesicles, a modification of the method of Klungsøyr (1987) was used. Fish were decapitated, the gills removed, and the gill arches cut off and rinsed in cold water. Each arch was blotted on filter paper to remove mucus, and the filament sheets were cut loose and rinsed in homogenizing buffer. Homogenizing buffer contained (concentrations in millimoles per litre) glycerol, 250; piperazine-*N*,*N*′-bis(2-ethanesulfonic acid) (PIPES), 2; ethylene glycol bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid (EGTA), 0.1; dithiothreitol (DTT), 1; and choline base to pH 7.5. The filaments were again blotted on paper, weighed, and homogenized as described (Klungsøyr 1987) in 10 volumes of homogenizing buffer. The homogenate was first centrifuged for 12 min at 1000 × *g* (pellet discarded) and then for 10 min at 9000 × *g* in a Sorvall centrifuge. This pellet, which contained most of the mitochondria, was saved and the supernatant was centrifuged in a Beckman L2-65 B centrifuge for 60 min at 57 000 × *g*. The sediment was resuspended in 0.5 mL of homogenizing buffer and layered on top of 5 mL of step buffer. The step buffer was a mixture of equal volumes of two solutions: (a) 38% sucrose, and salts and buffer as in homogenizing buffer, and (b) 120 g of glycerol (87%), 80 g of water, and salts and buffer as in homogenizing buffer based on the volume of glycerol plus water. Centrifugation was carried out in a Beckmann 50 Ti (angle) rotor in 10-mL capacity tubes at 82 000 × *g* for 60 min. The interface layer was collected with most of the turbidity

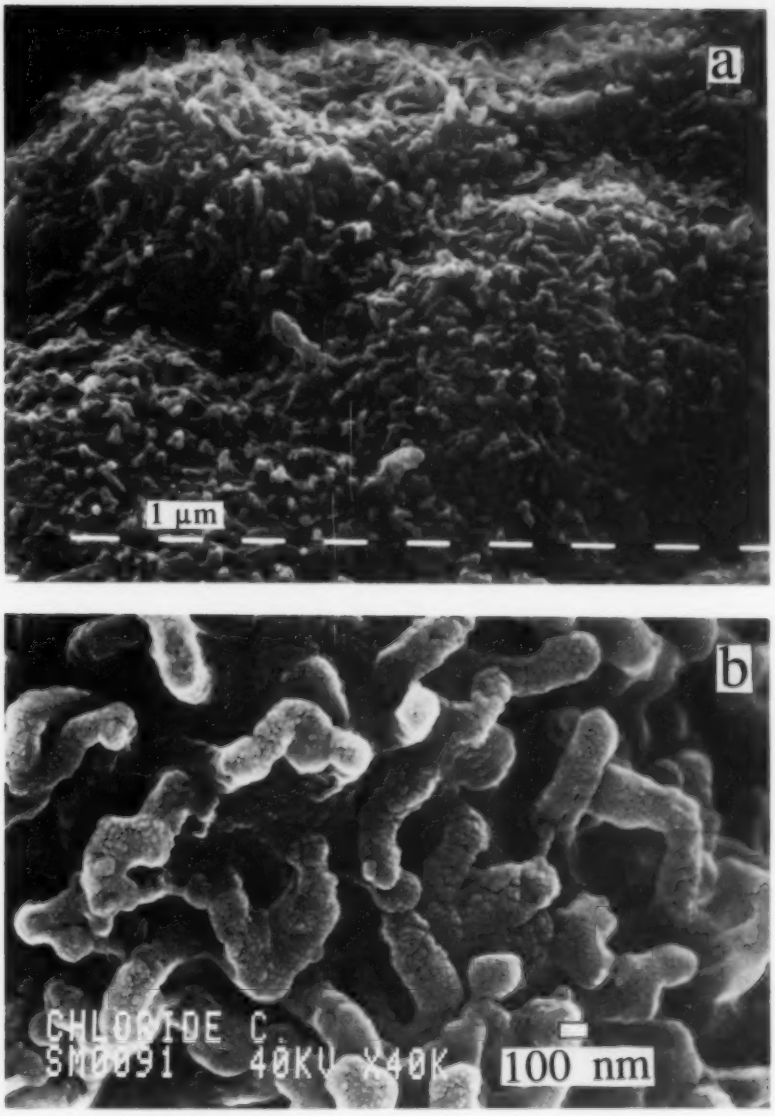

FIG. 3. (a) Chloride cells on the surface of gill lamella. This control animal had been taken directly from the storage tank and quickly decapitated. Magnification 8000×. (b) Microvilli from Fig. 3a at 40 000×.

(1.5 mL) and diluted to 10 mL with homogenizing buffer. The vesicles were then collected by centrifugation for 30 min at 82 000 × *g*. The pellet was suspended in component b of the step buffer and frozen in liquid nitrogen.

The vesicle suspension was thawed for use and diluted to 10 mL with magnesium-free running buffer. This contained (concentrations in millimoles per litre) KCl, 130; PIPES, 2; EGTA, 0.1; and KOH to pH 7.0.The vesicles were collected by centrifugation at 57 000 × *g* for 60 min and resuspended in magnesium-free running buffer to 3–5 mg protein·mL^{-1}.

Frozen mitochondrial pellets were thawed and resuspended in magnesium-free running buffer to which was added 1 mM DTT. The suspension, in a 15-mL centrifuge cup cooled in ice, was treated with a Branson sonifier (microtip) at setting 6 by the 20% pulsed mode for 10 min. The treated suspension was centrifuged at 12 000 × *g* for 10 min and the supernatant further centrifuged for 60 min at 57 000 × *g* for 60 min. The pellet (submitochondrial particles) was suspended in magnesium-free running buffer containing 1 mM DTT to a protein concentration of approximately 10 mg·mL^{-1}.

To measure proton uptake in vesicles or submitochondrial particles, the following assay mixture was used. To magnesium-free running buffer was added 5 mM magnesium sulfate and 10 mM phospho(enol)pyruvate, and pH was adjusted back to 7.0. Further additions were 1 µM acridine orange, 2 µM valinomycin from an ethanolic stock solution, and 30 units pyruvate kinase·mL^{-1} in glycerol solution. Solutions (20 nM) of *N*-methylmaleimide (NEM) were prepared daily in magne-

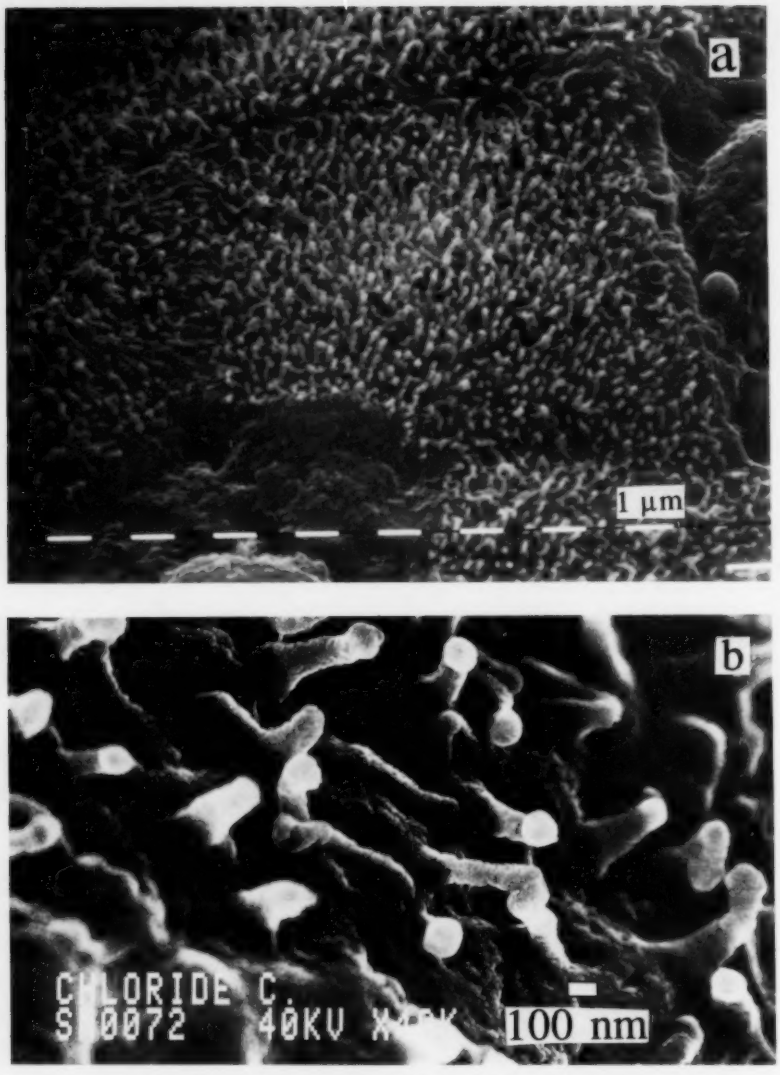

FIG. 4. (a) Chloride cells on the surface of gill lamellae. This animal had been kept in water that was bubbled with air containing 5% carbon dioxide for 2 × 40 min. Magnification 8000×. (b) Microvilli from Fig. 4a at 40 000×.

sium-free running buffer. When appropriate, oligomycin was added from an ethanolic solution to a final concentration of 20 $\mu g \cdot mL^{-1}$.

Acridine fluoresence was measured with an Aminco spectrofluorometer equipped with a strip chart recorder. Exciting wavelength was 490 nm and emitted light was measured at 520 nm. In a specially adapted microcuvet, 0.4 mL of assay mixture was mixed with 50 µL of vesicle suspension. The fluoresence intensity was adjusted to 80–90% of maximal recorder deflection, and after the baseline was established the uptake was started with 10 µL of 100 mM MgATP. In experiments with NEM inhibition (at 1 mM), the vesicle suspension was preincubated at room temperature with the inhibitor for 10 min.

Ten vesicle preparations were used for these experiments, each made from the gills of eight fish. Each preparation was tested for NEM inhibition prior to experiments.

Gills for electron microscopy were obtained from hypercapnic and control fish. Tap water was adjusted to pH 8 with calcium hydroxide solution (approximately 0.1 mM), and the water in the bucket containing the experimental fish was bubbled with air containing 5% carbon dioxide. After 30 min of this treatment the fish were divided into two groups and transferred to separate small glass containers. Each contained 800 mL of the same kind of water to which had been added markers for detection of endocytosis: iron-labelled latex microspheres or iron-labelled dextran. One container was bubbled with regular room air, while the other received the air containing the 5% carbon dioxide. The fish were kept under these conditions for 40 min more. In the container with carbon dioxide the pH was then 5.4, and in the container with air the pH was 6.8.

The fish were killed by decapitation, the gill arches were cut

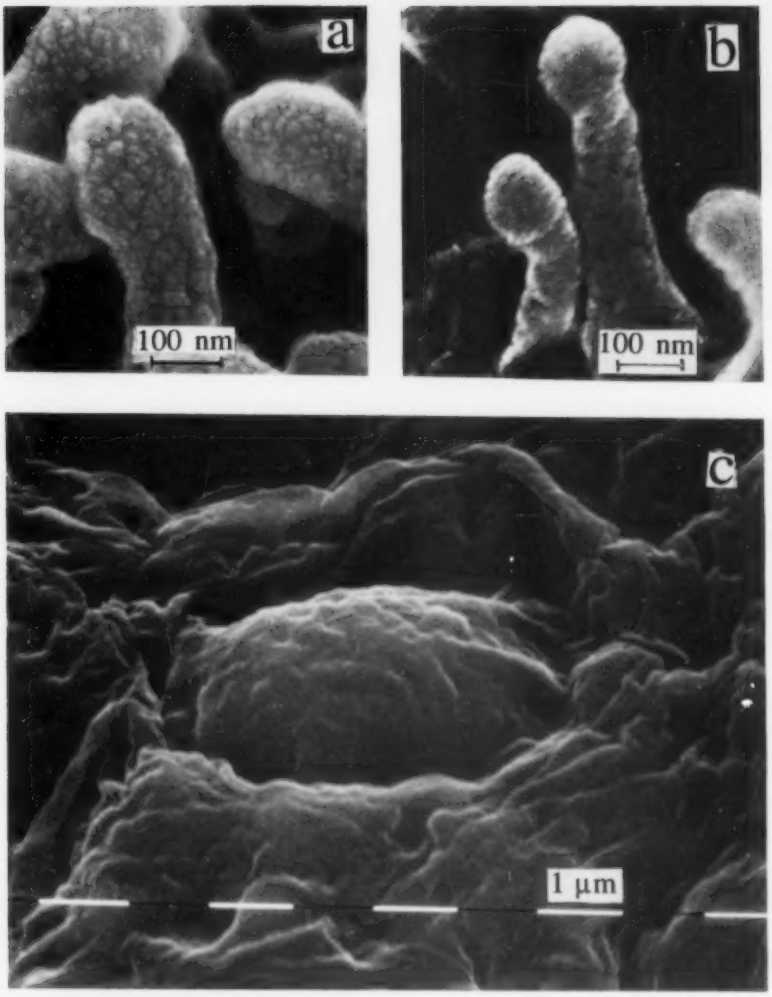

FIG. 5. Microvilli from Fig. 4a at (a) 120 000× and (b) 100 000×. (c) Chloride cell on the surface of gill lamellae. Magnification 1350×. This animal had been kept for 40 min in water that was bubbled with air containing 5% carbon doixide and then changed to laboratory air and the bubbling continued for 40 min more.

out, given a short rinse in ISOTON II (Coulter Electronics), and fixed for 4 h in the same solution by gradually adding 25% glutaraldehyde until a concentration of 2% was reached. The temperature was kept at 4°C. Fixed tissues were thoroughly rinsed in ISOTON II. Subsequently, freeze-substitution was carried out at −26°C for 3 d. The fine structure of the cell surfaces was preserved by the critical point method. Specimens mounted on stubs were coated with gold–palladium and examined either in a Phillips PSEM 500 scanning electron microscope operated at 25 kV or, for magnifications exceeding 10 000 × in a JEOL JEM-1200 CX electron microscope equipped with a lathanum hexaboride thermionic electron emitter operated at 40 kV.

For energy-dispersive X-ray microanalysis, epon sections, 60 nm thick, were mounted on copper grids and examined in a JEOL JEM-100 CX combined transmission and scanning microscope. The microscope was equipped with a Kevex lithium-drifted silicon energy-dispersive X-ray detector connected to a Kevex Quantex-Ray/uX 7000 system. Electron beam diameter was 8 nm, accelerating voltage was 40 kV, incident angle was 60.0°, and effective take-off angle was 28.2°. The collimator diameter was 2 mm and specimen distance was 12 mm.

Results

Proton Transport

In agreement with earlier observations (Klungsøyr 1987), oligomycin had little effect upon ATP-driven proton uptake by the vesicles (Fig. 1a). NEM had little effect on the ATPase activity of the vesicles (results not shown), but the proton transport was completely inhibited by 1 mM of the SH-reagent (Fig. 1a).

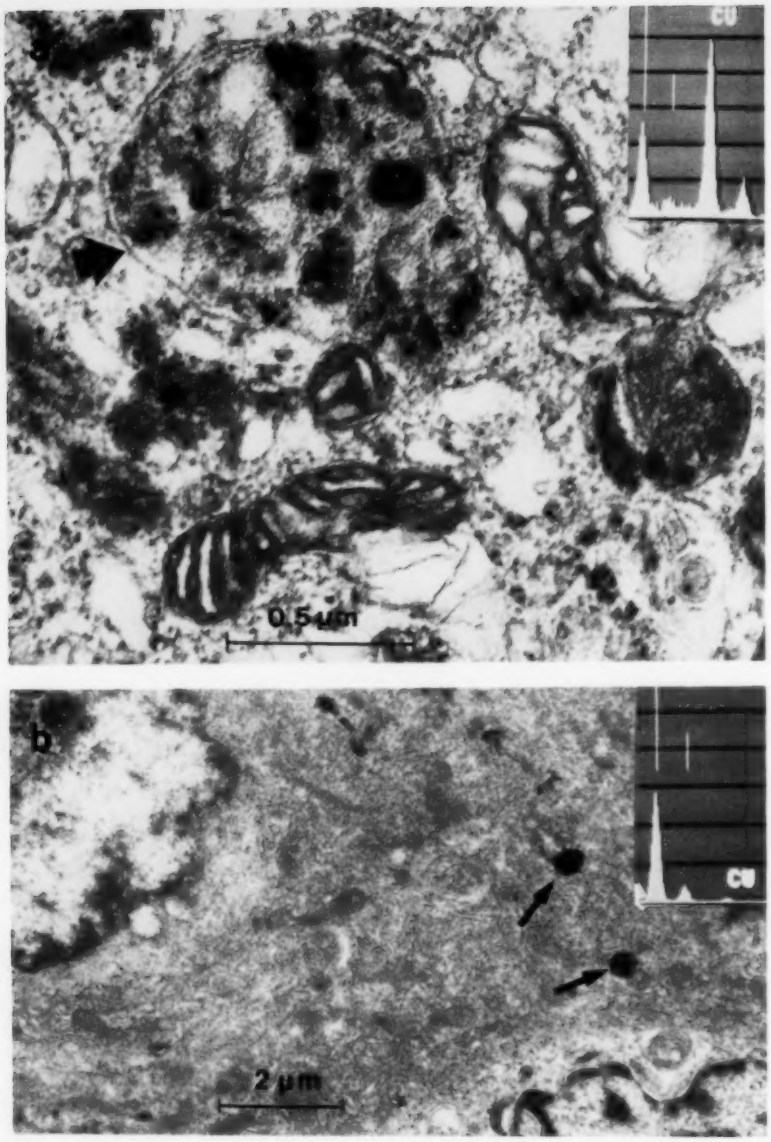

FIG. 6. (a) Transmission electron microscopy of gill tissue from a fish that was incubated in water containing iron-labelled dextran. The fish was pretreated for 30 min with air containing 5% carbon dioxide and then given laboratory air for the next 40 min. Note the membrane-enclosed vesicle that contains electron-dense material (arrow). Magnification 60 000×. Inset: Micro-X-ray spectroscopic identification of iron in the vesicle. The long line points to the iron α-peak and the short line to the iron β-peak. The copper peak originates from grid material. (b) As in Fig. 6a, except that the fish was incubated in water to which was added a suspension of iron-labelled latex microspheres. Magnification 10 000×. Inset: Micro-X-ray spectroscopic identification of iron in a microsphere observed within a cell. Peak identification as in Fig. 6a.

Submitochondrial particles demonstrated a very rapid ATP-dependent proton uptake (Fig. 1b). This uptake seemed to also have a slower phase. Oligomycin inhibited the rapid phase completely, while 1 mM NEM seemed to inhibit the slow phase, even though the preparation contained 1 mM DTT. The rapid phase was unchanged in the presence of 1 mM NEM.

Even in the presence of 1 mM DTT, 1 mM NEM gave considerable if incomplete inhibition of the proton transport into the ATPase vesicles (Fig. 1c). If the NEM concentration was increased to 3 mM, complete inhibition was again observed.

In experiments with 0.1 mM vanadate as a possible inhibitor of the proton transport into the vesicles, we found no detectable inhibition of the uptake (results not shown).

Nonspecific Endocytosis

Gill epithelium of control fish, i.e., fish that had been taken directly from the storage aquarium, had epithelial cells with microridges and "pits," probably made by emerging chloride cells (Fig. 2). The epithelium from the control fish also contained numerous chloride cells on the lamellar surface, densely covered with microvilli that were in a relaxed, not fully extended, state (Fig. 3).

The gills of fish from water that was bubbled with air containing 5% carbon dioxide for periods of 60–80 min had on much of their lamellar surface almost a continuous layer of cells that were studded with erect microvilli (Fig. 4, 5a, and 5b). Such structures are most likely to belong to chloride cells.

When the fish were treated first with air containing carbon dioxide, and then given ordinary air afterwards, the gill epithelium contained few cells with microvilli. The lamellar structure had many cells that were apparently in the act of "withdrawing from the surface" and could best be described as chloride cells without microvilli (Fig. 5c).

Uptake of iron-labelled dextran or iron-containing latex microspheres was not observed in gill epithelial cells from fish that were treated continuously with air containing carbon dioxide. If, however, the fish were pretreated with air containing carbon dioxide, and then given ordinary room air, and at the same time given markers in the water, uptake into gill cells took place. This could be demonstrated by transmission electron microscopy using iron-labelled dextran as a marker (Fig. 6a), and the iron could be definitely identified by X-ray microspectrography. Similar results were obtained with iron-labelled microspheres (Fig. 6b).

Discussion

Elimination of the major acid–base couple produced during fish metabolism, carbon dioxide and bicarbonate, can apparently be achieved mostly by transport of diffusible species, carbon dioxide and ammonia base. The remaining problems are those of homeostasis and regulation. We have postulated that in freshwater, fish excess acidity is eliminated by a proton pump (Klungsøyr 1987).

The experiments presented in this paper further document the existence of a proton pump in vesicular gill preparations. This pump is different from that found in submitochondrial particles and resembles the system that has been studied in mammalian kidney tubules and bladder of turtles (Schwartz and Al-Awqati 1986). We have demonstrated that the gill ATPase transports protons by a vehicle that is insensitive to oligomycin at concentration that completely inhibit the mitochondrial system. The gill pump is completely inhibited by NEM, but not by vanadate.

We know very little about how the gill proton-pumping ATPase is controlled on a molecular level, except that it has a low pH optimum (Klungsøyr 1987). In this work, we have attempted to find evidence for a cellular regulation of the pump density in the cell membrane, analogous to what is found in the turtle bladder and the mammalian kidney tubule (Schwartz and Al-Awqati 1986). It has been demonstrated that the number of chloride cells in the gill epithelium and the density of microvilli on chloride cell surfaces are influenced by the salt concentration in the water (Hossler 1980; Laurent and Dunel 1980).

Our results with rainbow trout demonstrated that intact fish treated with carbon dioxide (hypercapnic fish) had a large number of chloride cells on the surfaces of the gill lamellae, and the apical surfaces of these cells were densely covered with microvilli. This happened very quickly because a treatment of only 40–80 min was sufficient to create this typical situation.

When the carbon dioxide treatment ceased, the gill chloride cell density decreased, and the microvilli disappeared from their surfaces. We assumed that during this period, there would be a rapid uptake of pinocytotic vesicles into the cells, and we showed that this was actually the case by two different methods: uptake of iron-labelled latex microspheres which were identified by micro X-ray emission and uptake of iron labelled dextran which was located within lysosomelike bodies by means of X-ray emission.

In conclusion, we have confirmed the existence of a proton-pumping ATPase in gill epithelial cells. This pump has an inhibitor sensitivity that is similar to the proton-pumping ATPases described in turtle bladder and mammalian kidney tubular preparations (Schwartz and Al-Awqati 1986). We have further shown that carbon dioxide acts as a powerful agent to increase the surface density of microvilli on chloride cells in the gill epithelium and apparently also the lamellar surface density of chloride cells. Removal of the carbon dioxide stress resulted in the disappearance of microvilli from the cell surface and uptake of macromolecules from the surrounding water into the gill epithelium chloride cells.

A preliminary report of this work has been given (Klungsøyr and Justesen 1987).

Acknowledgements

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Relationship between Growth and Total Nucleic Acids in Juvenile Pink Salmon, *Oncorhynchus gorbuscha*, Fed Crude Oil Contaminated Food

Shiao Y. Wang

Department of Biological Sciences, University of Southern Mississippi, Hattiesburg, MS 39406-5018, USA

and Judith L. Lum, Mark G. Carls, and Stanley D. Rice

Auke Bay Laboratory, National Marine Fisheries Service, NOAA, 11305 Glacier Highway, Juneau, AK 99801-8626, USA

Wang, S.Y., J.L. Lum, M.G. Carls, and S.D. Rice. 1993. Relationship between growth and total nucleic acids in juvenile pink salmon, *Oncorhynchus gorbuscha*, fed crude oil contaminated food. Can. J. Fish. Aquat. Sci. 50: 996–1001.

Total nucleic acids of juvenile pink salmon, *Oncorhynchus gorbuscha*, fed crude oil contaminated food were analyzed to determine if nucleic acid measurements can be used to evaluate growth of fish collected at oil spill sites. In general, the nucleic acid concentration (micrograms per milligram dry weight) of salmon fry fed food contaminated with either 0.37 or 2.78 mg crude oil/g food was not significantly affected. However, RNA concentration of fry fed food contaminated with 34.83 mg/g was reduced whereas DNA concentration increased. Results over 8 wk indicate decreased protein synthesis and cell content but maintenance of cell integrity in these fish. Growth was inversely related to the level of crude oil contamination in the food. The significant correlations between measured growth and RNA/DNA ratios and RNA contents (micrograms RNA per millimetre fork length) suggest that nucleic acid measurements can be used to compare growth of fish collected from the field.

Les acides nucléiques totaux de saumons roses juvéniles, *Oncorhynchus gorbuscha*, exposés à du pétrole brut par l'intermédiaire de leur alimentation ont été analysés pour déterminer si les mesures portant sur l'acide nucléique peuvent être utilisées pour évaluer la croissance de poissons prélevés sur les lieux où s'est produit un déversement de pétrole. En général, la concentration d'acide nucléique (microgrammes par milligramme en poids sec) chez des alevins de saumon exposés à des aliments contaminés par 0,37 ou par 2,78 mg de pétrole brut/g d'aliment n'a pas été modifiée de manière statistiquement significative. Toutefois, la concentration d'ARN chez des alevins exposés à des aliments contaminés avec 34,83 mg/g a diminué, tandis que celle de l'ADN a augmenté. Les résultats après huit semaines d'exposition indiquent une diminution de la synthèse protéique et de la teneur cellulaire en protéines, mais le maintien de l'intégrité cellulaire chez ces poissons. La croissance était inversement proportionnelle à la concentration de pétrole brut dans les aliments. Les corrélations statistiquement significatives entre la croissance mesurée et les rapports ARN/ADN et la teneur en ARN (microgrammes d'ARN par millimètre de longueur à la fourche) laissent entendre que les mesures portant sur les acides nucléiques pourraient être utilisées pour comparer la croissance des poissons prélevés sur le terrain.

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Growth inhibition of salmonids as a result of exposure to the water-soluble fraction of crude oil (Moles and Rice 1983) or ingestion of petroleum-contaminated prey (Schwartz 1985) has been demonstrated in laboratory studies. Reduction in growth is a sensitive indicator of exposure effects because sublethal exposures cause metabolic changes and increased energy demands on the fish (see review by Rice 1985). After the *Exxon Valdez* oil spill in Prince William Sound, Alaska, in March of 1989, growth of pink salmon, *Oncorhynchus gorbuscha*, fry in situ was probably affected because fry caught in oiled areas were smaller and had lower apparent growth rates than fry caught in clean areas (A. Wertheimer, Auke Bay Laboratory, P.O. Box 210935, Auke Bay, AK 99821, USA, personal communication). However, definite conclusions on the growth of wild salmon fry were difficult to make because their time of entry into seawater, initial size, and residence time in oiled waters were not known. The need for an alternative indicator of growth in wild fish stimulated the present research.

Because growth is the result of different biochemical processes, measurements of selected biochemical markers should be useful as early indicators of perturbation in the growth process. One such measurement that is correlated with growth is the RNA/DNA ratio (see review by Bulow 1987). Since RNA is necessary for the translation of DNA, its concentration is a reflection of protein synthesis activity. The quantity of DNA per cell is usually constant and thus, DNA level is correlated with cell number. The RNA/DNA ratio then serves as an index of cellular protein synthesis activity and growth.

Changes in environmental conditions that would cause decreased growth in fish have been shown to alter their RNA/DNA ratios. RNA/DNA ratios decreased as a result of exposure to various toxicants in the fathead minnow, *Pimephales promelas* (Barron and Adelman 1984), and nutritional stress in the Atlantic cod, *Gadus morhua* (Lied et al. 1983), rainbow trout, *Oncorhynchus mykiss* (Jurss et al. 1986, 1987), Atlantic herring, *Clupea harengus*, and turbot, *Scophthalmus maximus* larvae (Clemmesen 1987, 1988), and common carp, *Cyprinus carpio* (Bastrop et al. 1991). RNA/DNA ratios also varied sea-

sonally in response to physiological condition and reproductive activity in bluegill, *Lepomis macrochirus* (Bulow et al. 1981). Correlation between growth and RNA/DNA ratio has been documented for the golden shiner, *Notemigonus crysoleucas* (Bulow 1970), several species of marine fish (Buckley 1984), larval striped bass, *Morone saxatilis* (Wright and Martin 1985), and saithe (pollock), *Pollachius virens* (Mathers et al. 1992). Although total nucleic acid measurements are useful for studying the effects of environmental factors on fish growth, their use in studying pollutants effects has been infrequent.

In the present study, RNA and DNA contents and concentrations of juvenile pink salmon fed crude oil contaminated food for 6 wk were analyzed. The goal of the study was to determine whether nucleic acid measurements can be used to evaluate the effects of crude oil on fish growth after an oil spill event. An effective biochemical indicator of growth would be one whose close correlation with measured growth can be demonstrated in the laboratory. The first objective was to determine the time course and pattern of quantitative changes in the nucleic acids of exposed fish. The second objective was to use results from the nucleic acid measurements to postulate cellular changes that take place in stressed fish.

Materials and Methods

Juvenile pink salmon, approximately 30 mm fork length, were obtained from the Auke Creek Hatchery, Juneau, AK. The fry were maintained in an 800-L cylindrical tank for 26 d prior to the start of the experiment. The tank received at approximately 20 L/min single-pass filtered seawater and the fry were fed Biodiet No. 2 starter feed (0.6–0.8 mm in diameter). The same feed was used later in the experiment. Four days before the start of the oil treatment, fry were randomly distributed into tanks for the five treatment groups. One group was fed uncontaminated food (untreated control), another was fed food exposed to the dichloromethane carrier solvent (treated control), and three groups were fed food contaminated with Alaska North Slope crude oil obtained from the Kenai Refinery of Chevron USA, Kenai, AK. The purpose of the treated control was to determine if dichloromethane, used as a carrier solvent to facilitate the mixing of crude oil in the food pellets, significantly degraded the nutritional quality of the Biodiet feed. Three target contamination levels of 0.8, 8.0, and 80 mg crude oil/g food were chosen based on the maximum amount of crude oil that the Biodiet feed can absorb (8% by weight). The actual amounts of crude oil in the contaminated food used were 0.37 ± 0.04 ($n = 2$), 2.78 ± 0.25 ($n = 7$), and 34.83 ± 5.40 ($n = 3$) mg crude oil/g food, hereafter referred to as low-, medium-, and high-dose food, respectively. The amount of crude oil in the food was determined by gas chromatography/mass spectrometry and gas chromatography/flame ionization detection for aromatic and aliphatic hydrocarbons, respectively. Three replicate tanks were used per treatment for a total of 15 tanks. Each of the 65-L rectangular ($53 \times 41 \times 30$ cm, L × W × H) tanks received a continuous supply of fresh seawater at a flow rate of 1.4 L/min. The seawater was filtered through a 25-μm filter to ensure that potential prey items were not introduced into the tanks. Water temperature was maintained at 7.8 ± 0.2°C.

To prepare oil-contaminated food, the appropriate amount of crude oil was dissolved in dichloromethane and added to 200-g batches of food pellets. The solvent was then removed using a rotary evaporator followed by 1 h of air drying. Contaminated food was prepared weekly as needed and stored frozen in glass jars sealed with Teflon-lined lids. Food for the untreated control was not modified in any way.

Fry were fed contaminated food for 6 wk. Uncontaminated food was fed for an additional 2 wk to examine possible recovery from the effects of oil exposure. Feeding rate, approximately 10% total body weight per day, was adjusted weekly based on the average weekly weight of fish in each treatment. Food was provided in excess, but the rate of ingestion was not quantified and uneaten food was removed twice daily. Fry to be analyzed for nucleic acids were narcotized with MS-222, weighed, measured for fork length, decapitated, eviscerated quickly, and frozen individually at −20°C. Fifteen fry from each tank, 45 from each treatment group, were sampled weekly. Fry that were fed low-dose food and those fed treated control food were analyzed at weeks 1 and 6 only. Fry that were fed control, medium-, and high-dose food were analyzed weekly. A total of 781 fry were analyzed.

The protocol for total DNA and RNA analysis was developed according to Munro and Fleck (1966). Frozen fry were blotted dry, weighed, and homogenized in 10 volumes of ice-cold distilled water using a Tekmar electric homogenizer. One-millilitre subsamples were dried at 80°C in preweighed aluminum pans for at least 8 h to determine the dry weight of each sample. Macromolecules including the nucleic acids were precipitated from 200 μL of the homogenate by adding 100 μL of 0.6 M and 500 μL of 0.2 M perchloric acid and incubating the mixture on ice for at least 10 min. The samples were then centrifuged for 10 min at 10 000 × *g* at 4°C. After discarding the supernatant, the pellet was washed twice with 750 μL of ice-cold 0.2 M perchloric acid. DNA content of the pellet was determined using the diphenylamine procedure (Burton 1956) according to Shatkin (1969). One millilitre of diphenylamine reagent along with 0.5 mL of 0.5 M perchloric acid was added to the pellet and incubated at 27 ± 1°C for 20 h. The DNA content of each sample was determined by comparing its optical density at 600 nm with those of standards prepared from salmon sperm DNA (Sigma Chemical Co.).

RNA content of the pellet was determined using a modified Schmidt-Thannhauser procedure recommended by Munro and Fleck (1966) in which ultraviolet absorption by RNA was measured. RNA was hydrolyzed by incubating the pellet in 1 mL of 0.3 M potassium hydroxide at 37°C for 1 h. After cooling the digest on ice, protein and DNA were precipitated by adding 0.5 mL of 1.5 M perchloric acid and incubating the mixture on ice for at least 10 min. The sample was centrifuged at 10 000 × *g* for 10 min at 4°C and the supernatant saved. The pellet was washed twice with 1 mL of 0.2 M perchloric acid. The supernatant from each of the two washes was saved along with the original (total = 3.5 mL). The RNA content of each sample was determined by comparing the optical density of the supernatant at 260 nm with those of standards prepared from yeast RNA (Sigma Chemical Co.).

DNA and RNA assays were done in duplicate. The specificity of the diphenylamine assay and the modified Schmidt-Thannhauser procedure for DNA and RNA, respectively, were tested using spiked samples. Test samples were spiked with extra protein (bovine serum albumin) and either yeast RNA or salmon sperm DNA. The assays were found to be specific.

Nucleic acid measurements were analyzed statistically in terms of both concentration and content. Concentration, defined as micrograms nucleic acid per milligram dry weight, refers to the amount of nucleic acid in relation to other tissue components. Content, defined as micrograms nucleic acid per millimetre fork length, refers to the amount of nucleic acid per unit

length and is independent of other tissue components. The distinction is important and their relevance to cellular changes in salmon fry that took place as a result of ingesting oil-contaminated food is discussed later.

Data were analyzed with nested ANOVA techniques (replicate tanks were nested within treatment) and variance was partitioned among experimental components using the Statistical Analysis System package (SAS Institute, Cary, NC, USA). Outliers, less than 3%, were detected and removed using the stem-leaf technique; statistical outcomes were not affected by outlier removal. The Dunnett multiple comparison test was used to test the significance ($p < 0.05$) of differences between means of each treatment and that of control fish.

Results

Ingestion of crude oil contaminated food had a significant effect on the nucleic acid level of salmon fry. After 1 wk, fry that were fed food contaminated with 34.83 mg crude oil/g (high-dose food) had a significantly lower RNA concentration (total RNA per milligram dry weight) but higher DNA concentration than control fish (Fig. 1a and 1b). RNA and DNA concentrations of fry fed treated control, low-, and medium-dose food were not significantly different from those of control fry. After 6 wk, the RNA concentration of fry fed high-dose food increased to a level equal to that of control fry. The RNA concentration of fry fed low-dose food remained equal to that of control fry, while the RNA concentration of fry fed treated control and medium-dose food was significantly lower than that of control fry. The DNA concentration of fry fed high-dose food increased and remained significantly higher than that of control fry.

Ingestion of clean food after 6 wk of feeding on oil-contaminated food caused compensatory RNA synthesis. After 1 wk of recovery during which all fry were fed clean food, the RNA concentration of those that had been fed high-dose food for 6 wk increased from 51.5 to 62.5 μg/mg dry wt which was significantly higher than that of control fry. Their DNA concentration decreased from 15.2 to 13.7 μg/mg dry wt but was still significantly higher than that of control fry. After 2 wk of recovery, the DNA concentration of fry fed high-dose food remained significantly higher than that of control fry, but the difference in RNA concentration was no longer significant. The RNA/DNA ratio of fry fed contaminated food was inversely related to the level of crude oil contamination during the first 6 wk (Fig. 1c). The RNA/DNA ratio of fry fed high-dose food remained consistently lower than that of control fry during the study.

Ingetion of crude oil contaminated food had clear dose- and time-dependent effects on the nucleic acid content (micrograms per millimetre fork length) of salmon fry. The RNA content of control fry increased steadily during the study (Fig. 2a). The RNA content of fry fed medium-dose food also increased but at a slower rate, and the difference from that of control fry was generally significant after the third week. The RNA content of fry fed high-dose food was significantly lower than that of control fry and remained unchanged until week 7, after 1 wk of recovery, when it increased dramatically from 20.5 to 33.6 μg/mm. The DNA content of control fry increased steadily during the study, while those of fry fed medium- and high-dose food remained essentially unchanged (Fig. 2b).

Growth, the increase in dry weight after 6 wk, took place in all treatments and was inversely related to the level of crude oil contamination in the food (Fig. 3). Growth was not signifi-

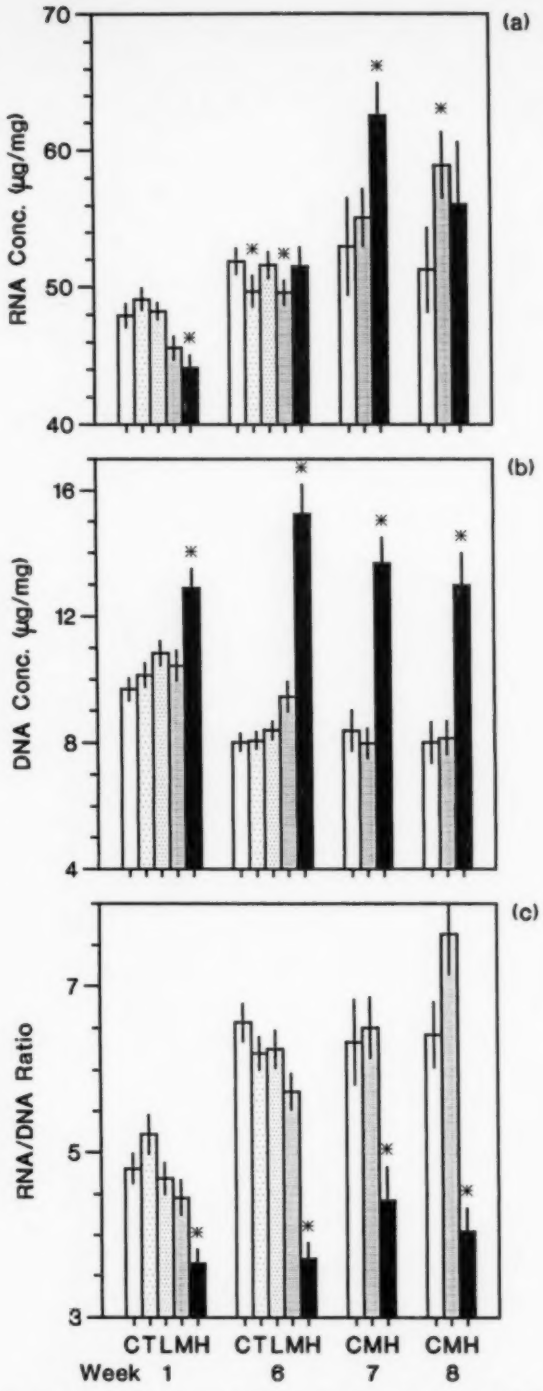

FIG. 1. Mean (a) RNA and (b) DNA concentrations and (c) RNA/DNA ratios of salmon fry fed crude oil contaminated food for 6 wk. Uncontaminated food was fed during weeks 7 and 8. Vertical lines represent standard error of the mean. Asterisks indicate significant difference from controls ($p \leq 0.05$). $n = 42$ for weeks 1 and 6 and $n = 13$ for weeks 7 and 8. C = control; T = treated control; L = low oil treatment (0.37 mg crude oil/g food); M = medium (2.78 mg/g); H = high (34.83 mg/g).

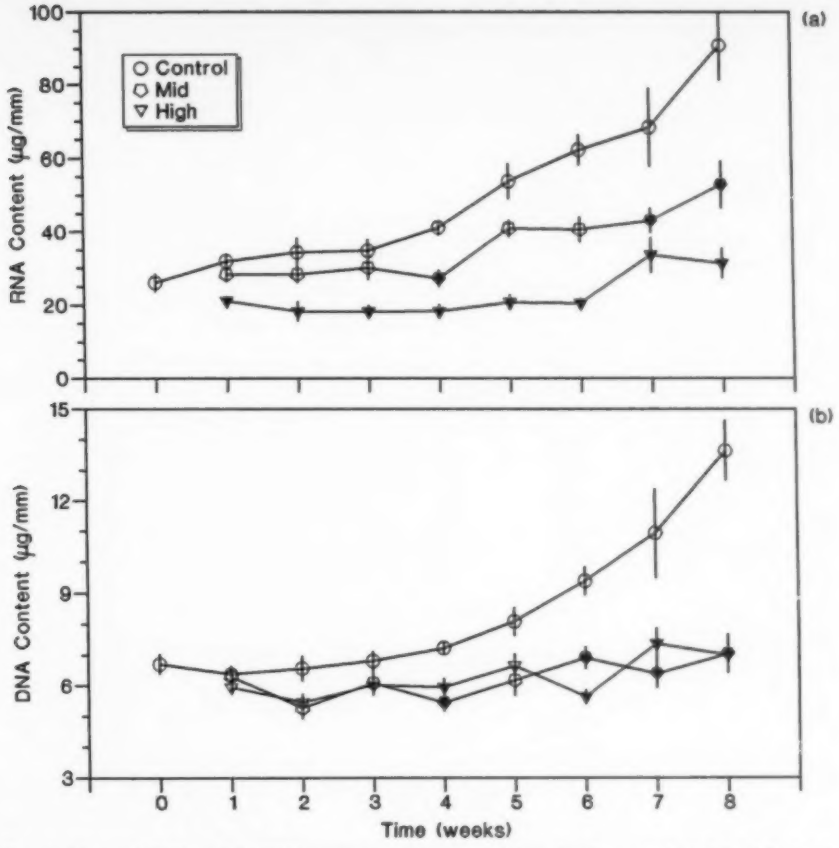

FIG. 2. Mean (a) RNA and (b) DNA content of salmon fry fed crude oil contaminated food for 6 wk. Uncontaminated food was fed during weeks 7 and 8. Vertical lines represent standard error of the mean. Solid symbols indicate significant difference from controls ($p \leq 0.05$). $n = 42$ for weeks 1 and 6 and $n = 12$ for the other weeks.

cantly correlated with RNA concentration (Fig. 4a), but it was with both RNA content and RNA/DNA ratio (Fig. 4b and 4c). Although the correlation was significant for both indices, growth was more closely correlated with RNA content than with RNA/DNA ratio. The linear relationship between growth and RNA content accounted for 93.3% of the variability in RNA content, while the relationship between growth and RNA/DNA ratio accounted for only 47.4% of the variability in RNA/DNA ratio.

Discussion

Our results allow us to postulate cellular changes in salmon fry that took place as a result of ingesting crude oil contaminated food. The significant decrease in RNA concentration after the first week in fry that were fed food contaminated with 34.83 mg crude oil/g indicates a decrease in protein synthesis that was probably caused by decreased feeding. Although daily food ingestion rate was not measured, several measurements of fecal production and stomach weight to body weight ratio clearly indicated a decrease in feeding by fry provided with oil-contaminated food. Due to the decreased feeding, less energy was available for growth, and the normal complement of cellular components for protein synthesis was not maintained. The level of ribosomal RNA and messenger RNA decreased relative to other cellular components, and a decrease in RNA concentration resulted. Subsequently, during the 6-wk when oil-contaminated food was fed to salmon fry, their RNA concentration increased to a level equal to that of control fry. This was probably due to the catabolism of cellular content. The level of RNA itself did not increase; instead, the proportion of RNA relative to other cellular components increased. It should be noted that growth in terms of both length and weight took place in all treatment groups. However, this was achieved at the expense of decreased cellular content among fish fed high-dose food.

Other published results support these suggestions. The difference in RNA concentration between fish that were nutritionally stressed and control fish depended on the duration of the stress. Lied et al. (1983) found decreased RNA concentration in the white trunk muscle of Atlantic cod that were starved for 8 d. Bulow (1970) reported that after 15 d, golden shiner fed 2% body weight per day had a lower RNA concentration than shiner fed 6% body weight per day. When this feeding regime was continued for 45 d, the difference in RNA concentration disappeared. Jurss and Bittorf (1990) found RNA concentration in the liver of immature rainbow trout that were fed a restricted ration for 44 d to be the same as that in trout fed ad libitum.

Our observation that DNA concentration increased in fry that

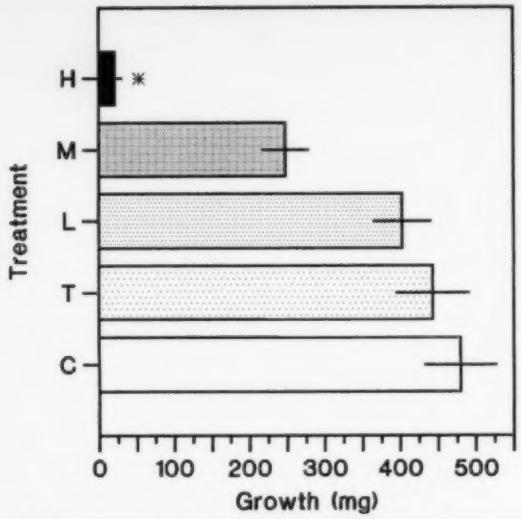

FIG. 3. Growth of salmon fry fed crude oil contaminated food (increase in dry weight after 6 wk). Asterisk indicates significant difference from controls ($p \leq 0.05$). C = control; T = treated control; L = low oil treatment (0.37 mg crude oil/g food); M = medium (2.78 mg/g); H = high (34.83 mg/g).

were fed oil-contaminated food further supports the suggestion found in the literature that decreased cell volume, as a result of the catabolism of cellular components, is a generalized response to long-term stress. Increased DNA concentration has been documented in the muscle and liver of bluegill during the spring and summer. The decrease in cell volume was probably a result of gonad maturation, spawning, and high maintenance energy requirements associated with high summer temperatures (Bulow et al. 1981). The number of cells in the liver of rainbow trout did not decrease after 4 wk of starvation, and the DNA concentration increased significantly (Jurss et al. 1986). In golden shiner, the difference in DNA concentration between fed and starved individuals increased over time (Bulow 1970). In general, it appears that by preserving DNA and cells, growth and recovery is favored when environmental conditions improve.

Increased growth and partial recovery were indicated in our study by the significant increase in RNA concentration and decrease in DNA concentration after fry that had been fed oil-contaminated food were fed clean food. We infer that increased RNA concentration reflects recent growth and can change significantly over a relatively short period.

We found a significant correlation between measured growth and RNA/DNA ratios in pink salmon fry that were fed food contaminated with different amounts of crude oil. The significant correlation provides support for the suggestion that the RNA/DNA ratios can serve as an index of fish condition and growth (see review by Bulow 1987). However, the amount of variation in the correlation may be a limiting factor in its usefulness as an indicator of actual growth (Bergeron et al. 1991). Miglavs and Jobling (1989) also questioned the use of RNA/DNA ratios to estimate fish growth after finding higher growth than that predicted by the RNA/DNA ratio during compensatory growth in Arctic char, *Salvelinus alpinus*. Instead, the inverse relationship between the level of crude oil contamination in the food and RNA/DNA ratios suggests that the ratio may be more useful for making relative comparisons of the growth status or condition among fish from different environ-

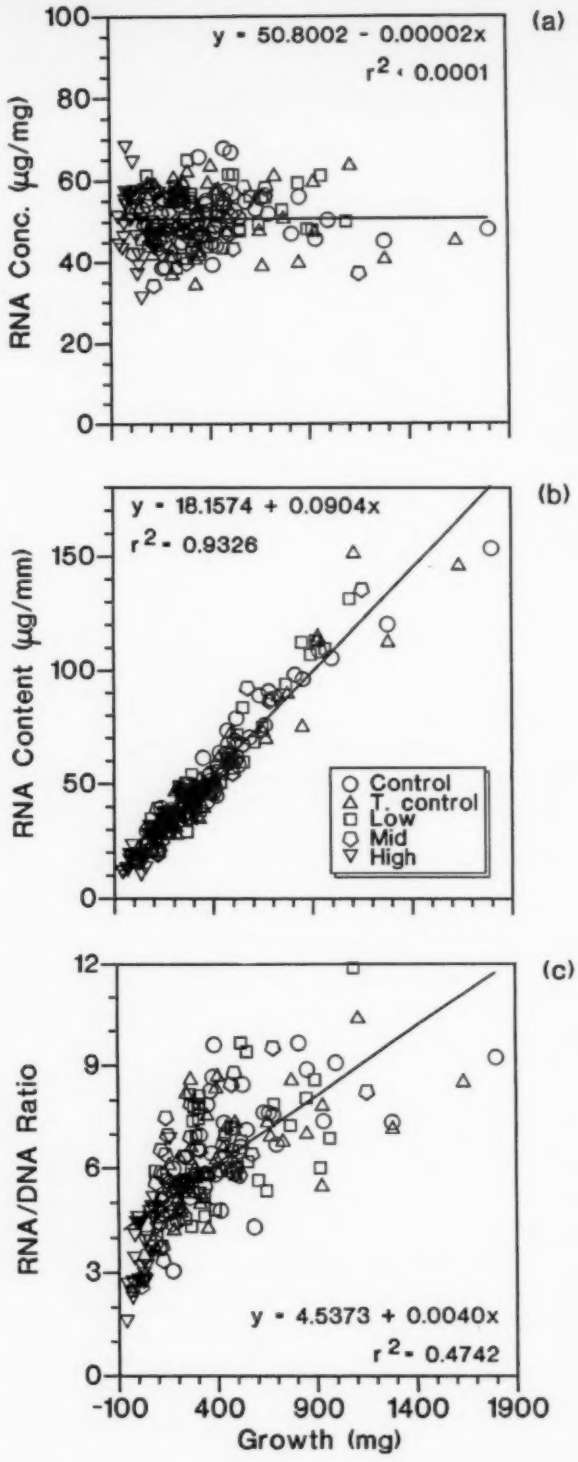

FIG. 4. Relationship between measured growth of salmon fry fed food contaminated with different levels of crude oil for 6 wk and indices of growth. (a) RNA concentration (n = 202); (b) RNA content (n = 202); (c) RNA/DNA ratio (n = 202).

mental conditions, rather than actual growth. The high degree of correlation between measured growth and RNA content (Fig. 4b) implies that RNA content may be a more useful indicator of growth than the RNA/DNA ratio (Fig. 4c).

The conceptual basis for using RNA content as an index of fish condition and growth is simple. Fish in good health and growing rapidly are robust and have high tissue mass to length ratios. Fish in poor condition are thin and have less tissue per unit length. Therefore, fish in good nutritional state and growing rapidly should have high total RNA to length ratios because RNA is an absolute requirement for protein synthesis and growth. The distinction between RNA concentration and content is important. RNA concentration refers to the amount of RNA in relation to other tissue components. RNA content refers to the amount of RNA per unit length and is independent of other tissue components. Fish in poor condition have less RNA than healthy fish. However, if the quantities of other tissue components are also lower, the amount of RNA in relation to other tissue components in slow-growing fish may be indistinguishable from that of fast-growing fish. The calculation of RNA content is independent of other tissue components. Therefore, slow-growing fish, having less RNA per unit length than fast-growing fish, would have lower RNA content, as demonstrated in the present study.

In order to use RNA content successfully as an index of condition and growth in fish, two precautions should be exercised. First, only eviscerated carcasses without heads should be used, or white muscle only if specimens are large enough. The white muscle is the most sensitive tissue to growth changes in terms of protein synthesis and degradation in the Atlantic cod (Houlihan et al. 1988). In addition, RNA content of liver and other internal organs can change in response to metabolic activities unrelated to growth (Bulow 1987). One possible reason for the high correlation coefficient between measured growth and RNA content in the present study was the use of fish from which the head and internal organs had been removed. The second precaution is that comparisons of RNA content should be made only among fish of the same species within a certain size range or life history stage. Because both growth rate and nucleic acid composition are likely to change with age and life history stage, it would not be appropriate to compare the RNA content of fishes differing widely in size or age. Because of the close correlation between measured growth and RNA content, further investigations on the use of RNA content as an indicator of condition and growth in fish are encouraged.

In conclusion, ingestion of food contaminated with 34.83 mg crude oil/g had significant time- and dose-dependent effects on the growth and nucleic acid level of pink salmon fry. A decrease in growth took place immediately, while catabolism of cellular content took place gradually over 6 wk. This trend was reversed during recovery when clean food was provided. Both RNA content and RNA/DNA ratio were significantly correlated with measured growth and thus can serve as indicators of growth of fish collected from the wild. However, estimations based on RNA content may be more reliable due to its tight correlation with measured growth.

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Pacific Salmon Production Trends in Relation to Climate

Richard J. Beamish and Daniel R. Bouillon

Department of Fisheries and Oceans, Biological Sciences Branch, Pacific Biological Station, Nanaimo, B.C., Canada V9R 5K6

Beamish, R.J., and D.R. Bouillon. 1993. Pacific salmon production trends in relation to climate. Can. J. Fish. Aquat. Sci. 50: 1002–1016.

Pink (*Oncorhynchus gorbuscha*), chum (*O. keta*), and sockeye salmon (*O. nerka*) represent approximately 90% of the commercial catch of Pacific salmon taken each year by Canada, Japan, the United States, and Russia. Annual all-nation catches of the three species and of each species, from 1925 to 1989, exhibited long-term parallel trends. National catches, in most cases, exhibited similar but weaker trends. The strong similarity of the pattern of the all-nation pink, chum, and sockeye salmon catches suggests that common events over a vast area affect the production of salmon in the North Pacific Ocean. The climate over the northern North Pacific Ocean is dominated in the winter and spring by the Aleutian Low pressure system. The long-term pattern of the Aleutian Low pressure system corresponded to the trends in salmon catch, to copepod production, and to other climate indices, indicating that climate and the marine environment may play an important role in salmon production.

Les saumons roses (*Oncorhynchus gorbuscha*), kéta (*O. keta*) et sockeye (*O. nerka*) représentent approximativement 90 % des prises commerciales de saumon du Pacifique faites par le Canada, le Japon, les États-Unis et la Russie. Les prises annuelles combinées de ces pays, de 1925 à 1989, ont montré des tendances parallèles à long terme aussi bien en ce qui a trait aux trois espèces ensemble que pour chacune des espèces prises individuellement. Les prises nationales ont présenté, dans la plupart des cas, des tendances semblables, mais moins prononcées. La grande ressemblance dans le profil des prises combinées de saumons roses, kéta et sockeye pour les quatre pays laisse entendre que des événements communs survenant sur une très grande superficie affectent la production du saumon dans le Pacifique Nord. Le climat dans la partie nord du Pacifique Nord est dominé au cours de l'hiver et du printemps par le système de dépression des Aléoutiennes. L'évolution à long terme du système de dépression des Aléoutiennes correspond aux tendances observées dans les prises de saumons, dans la production de copépodes et dans d'autres indices climatiques, indiquant que le climat et l'environnement marin peuvent jouer un rôle important dans la production de saumons.

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Pink salmon (*Oncorhynchus gorbuscha*), chum salmon (*O. keta*), sockeye salmon (*O. nerka*), coho salmon (*O. kisutch*), and chinook salmon (*O. tshawytscha*) contribute to major commercial fisheries in the North Pacific Ocean and adjacent seas. Approximately 90% of the commercial catch taken each year by Canada, Japan, the United States, and Russia is composed of pink, chum, and sockeye salmon (Table 1). These catches fluctuated throughout this century, with high catches occuring in the late 1930s and 1980s. Changes in the abundance of Pacific salmon are generally considered to be related to changes in the abundance of spawners (Ricker 1954), often referred to as escapement (fish that escape the fishery). As a consequence of Ricker's (1954) studies, management strategies for salmon commonly attempt to maximize sustainable production by achieving optimal spawning escapements. Because a large percentage of salmon returning to spawn are caught in the fishery, the abundance of spawners is generally smaller than catch.

The anadromous life cycle of Pacific salmon makes them vulnerable to overfishing in both the marine and freshwater environments and to degradation of freshwater habitat. Reductions in the quality of freshwater habitat by pollution and reductions in the amount of available habitat throughout this century might be expected to have caused a long-term decline in the survival of salmon eggs and fry. Such a reduction in survival, combined with high exploitation rates, should lead to long-term declines in salmon catch, but the total all-nation catch actually increased beginning in the late 1970s. The dramatic increases in catch that occurred suggested to us that the marine environment was closely linked to the increases in salmon production. However, the survival of salmon in the marine environment is particularly difficult to study and total mortality can exceed 95% (Foerster 1968; Heard 1991; Salo 1991). Much of this mortality is believed to occur shortly after salmon enter salt water, decreasing as the fish grow larger.

The marine distribution of Pacific salmon differs among species (Fig. 1) with overlapping distributions of Asian and North American stocks in the northern North Pacific Ocean. The cli-

TABLE 1. Average catch (1000 t) of Pacific salmon (see text for data source).

	United States	Japan	Russia	Canada	Total
Pink salmon[a]	77.2	68.2	71.1	20.8	237.3
Chum salmon[a]	32.1	69.1	43.0	18.2	162.4
Sockeye salmon[a]	57.3	17.6	8.0	14.0	96.9
Coho salmon[b]	16.5	5.4	4.0	10.7	36.6
Chinook salmon[b]	14.4	0.9	1.5	6.3	23.1
Total	197.5	161.2	127.6	70.0	556.3

[a]From 1926 to 1989.
[b]From 1952 to 1989.

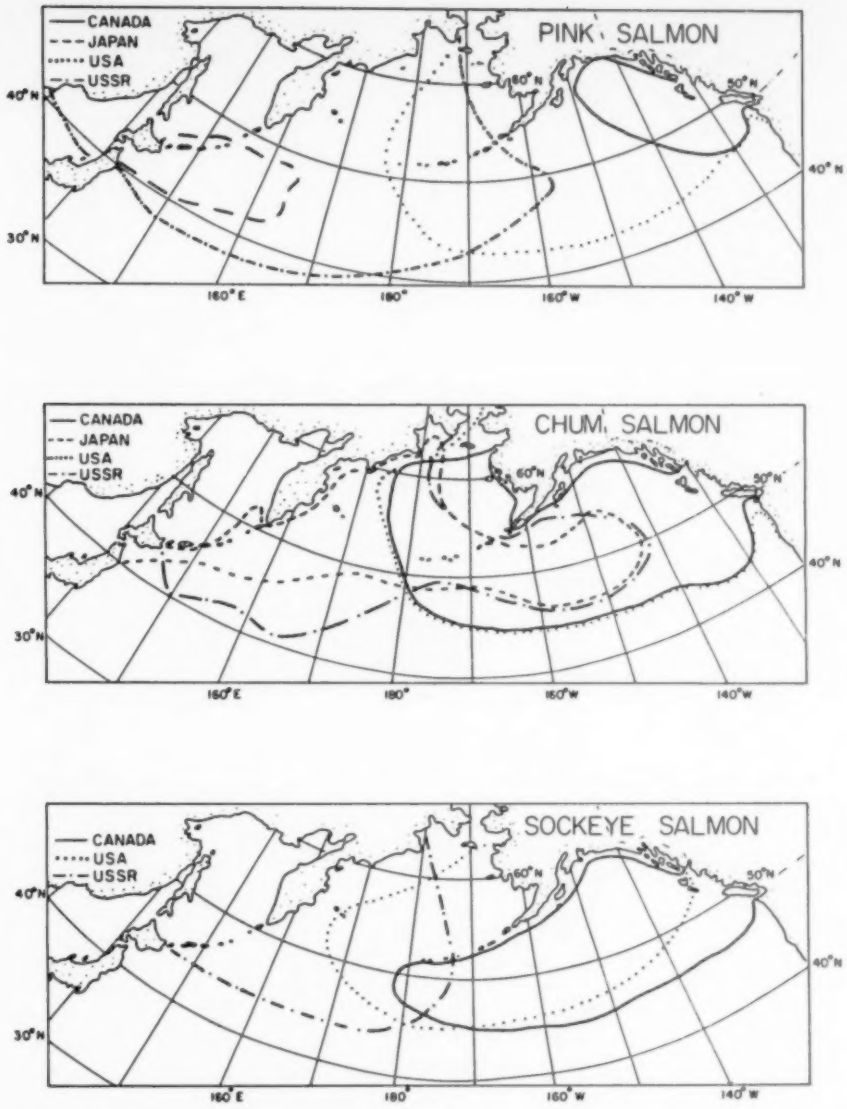

FIG. 1. Known limits of ocean distribution of pink, chum, and sockeye salmon as determined from tag recoveries, 1956–89. Data from Margolis (1990), Neave et al. (1976), and Takagi et al. (1981). Canadian chum salmon distribution includes chum from the Yukon River, Yukon. There are no significant numbers of sockeye that return to Japan.

mate of this region from late in the year to early in the next year is dominated by the Aleutian Low pressure system (Fig. 2). Hence, climate–ocean events in the central northern North Pacific Ocean can affect both Asian and North American salmon stocks and can also influence coastal regions (Royer 1982; Niebauer 1988; Ebbesmeyer et al. 1989). The overlapping distribution of salmon and the potential wide-ranging effects of climate and oceanographic conditions suggest that the changes in production of salmon originating from the four major salmon-producing countries might be affected by common events in the marine environment. Therefore, in this paper, we examine the trends in salmon production in relation to large-scale environmental changes in the northern North Pacific Ocean.

Methods

We did not include coho and chinook salmon in our analysis because catches of these species represent a small proportion of the commercial catch (~10%, Table 1), and in recent years the catches in the recreational fishery have complicated the use of commercial catch as an estimator of salmon production. Commercial catch was used as an index of salmon production for pink, chum, and sockeye salmon. Total returns (catch plus escapement) should be used, but it was not possible to obtain accurate estimates of escapement of all stocks, from all countries, for the time series. Catch is an acceptable index of abundance because a large percentage of salmon stocks is caught in

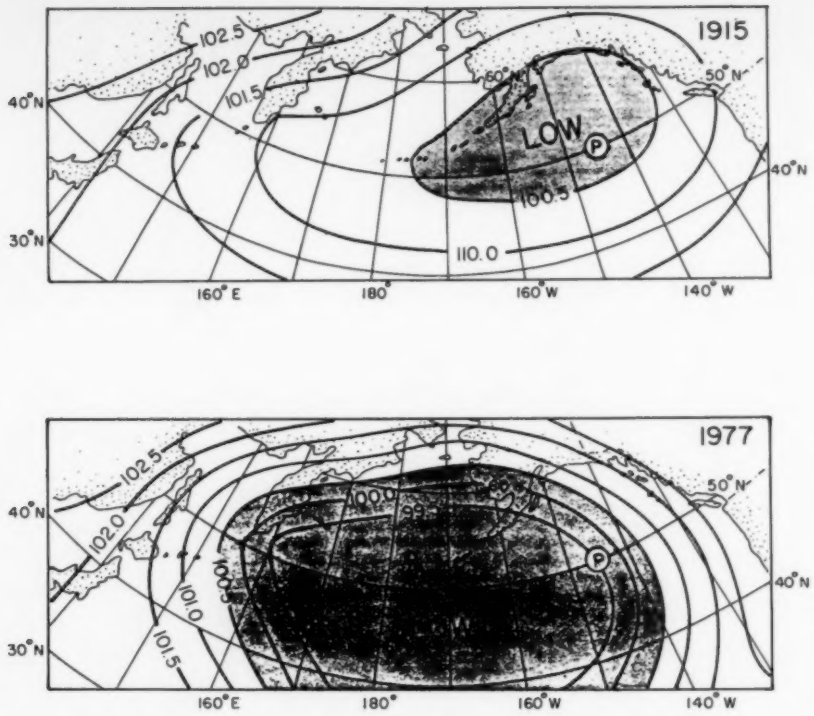

FIG. 2. Examples of a weak Aleutian Low pressure system for January 1915 and a typical intense Aleutian Low for January 1977. Sea surface pressure gradients are in kilopascals. P is the approximate location of Ocean Station P.

the coastal fisheries (exploitation rates typically range from 65 to 85%). We realize that catch may not always be a good indicator of abundance, but for larger aggregates of stocks, we propose that catch trends represent the abundance trends of the total aggregate.

We assembled catch data by country and by species. Our time series started in 1925 because some data sets were missing for years before 1925, and ended in 1989 because data were incomplete after this date. Catches for Canada, Japan, and the United States were coastal catches obtained from the publications of the International North Pacific Fisheries Commission (INPFC) (1977–89, 1979). Russian catches of pink, chum, and sockeye salmon from 1940 to 1974 (Table 2) were obtained from the INPFC (1979). Russian catches from 1925 to 1939 and from 1975 to 1989 (Table 2) were obtained from unpublished catch statistics. Catches of pink salmon in Russia were not available for 1925.

The Japanese high-seas driftnet fishery for salmon intercepted salmon produced in other countries (Shepard et al. 1985). These interceptions affect our analyses when we look at national catch trends and when we examine total salmon production. National trends were difficult to correct, but by combining Japanese and Russian catches into an Asian data base and Canadian and United States catches into a North American data base and then correcting for lost production to the North American data base resulting from the Japanese high-seas salmon driftnet fishery, we were able to compare the production trends of salmon produced in Asia and North America. We used estimates of the number of North American salmon intercepted in the high seas driftnet fishery from 1956 to 1975 from Fredin (1980). Numbers were converted to weight using the annual mean weight of pink, chum, and sockeye salmon caught in the Japanese high-seas driftnet fishery (INPFC 1979). We subtracted this weight from the Asian catch and added it to the North American catch after correcting for lost production using scenario 2 in the method estimating lost production for the all-nation catches that follows.

Calculation of Lost Production due to Japanese High-Seas Driftnet Fishing

Using total North Pacific Ocean coastal salmon catch as an index of total production introduces a bias because lost production resulting from high-seas driftnet fishing is not incorporated in the estimate. Salmon caught in Japanese high-seas mothership and land-based driftnet fisheries are not given the opportunity to put on the substantial growth that typically occurs in the last year of life (Ricker 1976). Before 1952 the Japanese fishery had virtually no impact on the production on North American salmon, but by the mid-1950s there was a well-developed high-seas fishery (Shepard et al. 1985). We estimated potential lost production starting from 1952 for pink, chum, and sockeye salmon and added these values to the known catch. Estimates made previously by Parker (1963), Fredin (1964), and Ricker (1964) used indirect estimates of lost growth (from the time the fish were caught on the high seas to the time they would have been caught in coastal fisheries) based on methods used to convert scale measurements to fish length (Ricker 1976). We estimated lost growth from annual high-seas and coastal mean weight per fish information (INPFC 1977–89, 1979). The mean midpoint of high-seas (mothership and land-

TABLE 2. Russian catches (1000 t) for pink, chum, and sockeye salmon from 1925 to 1989. Data from 1925 to 1939 and from 1976 to 1986: M.Y. Kazarnovsky, VNIRO, Moscow, Russia, personal communication; data from 1940 to 1975: INPFC 1979; data from 1987 to 1989: A.I. Chigirinsky, TINRO, Vladivostok, 690600, Russia, personal communication.

Year	Pink	Chum	Sockeye	Total	Year	Pink	Chum	Sockeye	Total
1925	—	37.9	15.6	—	1958	38.4	27.7	1.0	67.1
1926	138.3	56.9	22.1	217.2	1959	47.1	38.2	4.0	89.3
1927	38.5	56.5	23.4	118.4	1960	19.6	43.3	4.0	66.9
1928	124.0	70.1	38.6	232.7	1961	30.3	36.4	7.8	74.5
1929	26.0	93.0	32.7	151.6	1962	16.3	34.0	4.7	55.0
1930	124.2	105.3	32.9	262.4	1963	35.7	33.6	3.4	72.7
1931	63.7	105.8	25.5	195.0	1964	14.6	25.4	2.7	42.7
1932	142.4	61.4	23.4	227.2	1965	47.9	31.5	4.2	83.6
1933	61.0	73.2	15.2	149.4	1966	20.7	27.6	3.7	52.0
1934	128.8	72.3	3.5	204.6	1967	50.7	20.6	3.0	74.3
1935	43.5	54.3	2.1	99.9	1968	16.3	13.7	2.2	32.2
1936	53.7	74.5	5.3	133.5	1969	63.4	5.9	1.6	70.9
1937	66.2	62.5	7.5	136.3	1970	16.2	12.4	4.5	33.1
1938	72.6	75.8	7.9	156.2	1971	58.4	10.5	2.2	71.1
1939	77.7	66.1	7.3	151.2	1972	20.4	5.1	1.0	26.5
1940	50.4	55.3	3.4	109.1	1973	66.4	5.2	1.7	73.3
1941	77.1	53.7	4.6	135.4	1974	32.3	7.1	1.1	40.5
1942	79.1	39.7	5.7	124.5	1975	69.0	7.8	1.5	78.3
1943	118.1	62.7	7.8	188.6	1976	53.5	15.6	1.1	70.2
1944	106.1	55.5	9.4	171.0	1977	114.4	20.5	1.9	136.7
1945	76.2	61.3	12.0	149.5	1978	55.8	27.2	3.3	86.4
1946	38.3	68.7	13.2	120.2	1979	100.3	19.8	2.8	122.9
1947	132.4	69.3	10.2	211.9	1980	77.4	14.0	3.9	95.2
1948	52.2	62.5	6.2	120.9	1981	86.7	12.4	3.8	102.9
1949	165.0	79.4	8.8	253.2	1982	47.7	11.4	2.3	61.3
1950	36.4	61.5	9.3	107.2	1983	104.3	18.7	4.3	127.3
1951	154.8	83.4	7.7	245.9	1984	54.7	13.7	6.2	74.6
1952	57.9	44.0	9.2	111.1	1985	95.2	20.7	9.6	125.5
1953	142.3	34.0	5.1	181.4	1986	40.8	27.5	8.2	76.5
1954	46.2	52.6	4.0	102.8	1987	101.2	27.3	11.2	139.7
1955	88.3	65.6	3.1	157.0	1988	40.3	26.8	9.3	76.4
1956	72.1	77.3	5.7	155.1	1989	152.7	24.5	10.6	187.8
1957	106.4	32.0	3.5	141.9					

based) catches was assumed to be July 1 of each year (INPFC 1979). The mean midpoint of coastal catches for each species was assumed to be August 1 for pink, September 1 for chum, and August 1 for sockeye salmon (Burgner 1991; Heard 1991; Salo 1991). Pink salmon caught on the high seas typically would have returned to spawn in the year of capture, while some of the chum and sockeye salmon caught in the high-seas fishery would not have spawned for one or several more years (Burgner 1991; Heard 1991; Salo 1991). Because our calculations used estimates of the average weight lost, we needed to estimate the average number of months of natural mortality that pink, chum, and sockeye salmon caught in the high-seas fishery would have experienced had they been able to migrate to the coastal areas. We used three scenarios to determine lost production: scenario 1, chum would experience an average of 2 mo of mortality and sockeye 1 mo; scenario 2, chum would experience an average of 14 mo of mortality and sockeye 13 mo; and scenario 3, chum would experience an average of 26 mo of mortality and sockeye 25 mo. For chum and sockeye salmon, scenarios 1, 2, and 3 refer to fish that all return to the coast either in the current year or the two subsequent years. Because pink salmon are caught in the high seas in the same year they spawn, we estimated an average of 1 mo of marine mortality between capture in the high seas and the coastal fisheries. These scenarios were used to describe the broad range of possible conditions of salmon returning to coastal waters. We assume that scenario 1 will overestimate lost production because of the low marine mortality assumption and that scenario 3 will underestimate production because of the high mortality assumption. Scenario 2, which has chum and sockeye salmon returning the year after high-seas capture, would probably be average conditions. Ricker (1976) concluded that the instantaneous natural mortality rate of sockeye salmon in their final year of life is about 0.015 per month and for pink and chum salmon is about 0.013, with mortality being higher in earlier years. We therefore calculated salmon production losses using a conservative value of M (monthly) = 0.02.

To calculate the potential numbers of salmon that would return to the coast in the absence of high-seas driftnet fishing, we applied the following formula to land-based and mothership high-seas driftnet catches for each species of salmon: $N_t = N \cdot e^{-(M \cdot t)}$ where N_t = total potential catch (in number) of coastal samon in the absence of high-seas fisheries, N = number of salmon caught in high-seas fisheries, M = monthly instantaneous natural mortality rate = 0.02, and t = number of months between catch of fish in the high-seas fisheries and anticipated catch in coastal fisheries as described in scenarios 1, 2, and 3. The total potential catch (N_t) was converted to biomass using the coastal mean weight estimates.

In addition to natural and fishing mortality, Ricker (1976) used noncatch mortality in his calculations of potential lost salmon production. Noncatch mortality includes those fish

injured in nets, or preyed upon while caught in nets, that subsequently die as a direct result of their net encounter. We do not incorporate noncatch mortality in our estimates of lost production because of the high level of subjectivity of such estimates. Nevertheless, noncatch mortality would result in higher levels of lost production than those we report here.

Aleutian Low Pressure Index

The Aleutian Low is usually centered over the Aleutian Islands and typically begins forming during the autumn months, intensifies during the winter months, and then breaks down by early summer. A weak or less intense winter pressure system is typical of the pattern observed in 1915, while the system present in 1977 is an example of an intense low (Fig. 2).

We produced mean seasonal sea level pressure maps for the North Pacific Ocean between 20 and 70°N latitude and between 120°E and 120°W longitude using sea level pressure data from the National Centre for Atmospheric Research, Boulder, CO, USA. Hamilton (1984) produced similar maps for the period 1939–82. For the winter (December, January, February) and the spring (March, April, May) of 1899–1990, we calculated the area (square kilometres) of the North Pacific Ocean covered by the Aleutian Low pressure system less than 100.5 kPa. This was accomplished by using a software package called COMPUGRD which produces two-dimensional maps of the region under investigation and calculates the area covered by chosen isobars. We used the sum of the winter and spring Aleutian Low values as an index of historical weather over the North Pacific Ocean to compare with salmon production trends. The Aleutian Low pressure indices were smoothed using a LOWESS smoother (Cleveland 1985) with a common band width, f, of 0.20.

The Southern Oscillation Index or El Niño events are frequently used as indices of climate change. The difference between the anomalies in sea level pressure at Tahiti minus the anomalies at Darwin is the Southern Oscillation Index and the extremes of the index are associated with El Niño (extreme negative anomaly) and La Niña (extreme positive anomaly) events. Instead of using these indices to compare climate events in the South and North Pacific oceans, we calculated the annual mean Darwin atmospheric sea level pressure for December–May. Mean monthly atmospheric sea level pressure at Darwin from 1900 to 1989 was obtained from the Climate Analysis Centre, World Weather Building, Washington, DC, USA.

Because temperature can affect fish production directly and indirectly, and because temperature is often used as an index of climate change, we wanted to compare salmon production trends with sea surface temperature trends in the northern North Pacific Ocean. In the absence of a time series of averaged sea surface temperature data for the northern North Pacific Ocean that corresponds to our time series for salmon production, we used the Northern Hemisphere mean surface temperature change developed by Hansen and Lebedeff (1987) as an index of sea surface temperature trends in the North Pacific Ocean. These land temperature data of Hansen and Lebedeff (1987) and Jones et al. (1986) show trends similar to the globally averaged sea temperature record (Reid 1987; Friis-Christensen and Lassen 1991).

We chose two methods to examine salmon production trends. First, we fit a LOWESS smoother (Cleveland 1985) to the catch data using a common band width, f, of 0.20. We also analyzed the data using a cumulative sum analysis, which plots the cumulative sum of the residuals around a mean value (Murdoch 1979; Campbell et al. 1991). This method provides a visual pattern of trends in the time series because changes in the slope are directly related to changes in the mean up to that point. Cumulative sum plots clearly identify inflection points in trends, and the position of the inflection points cannot be adjusted by manipulating the analysis as can be done by some other smoothing methods.

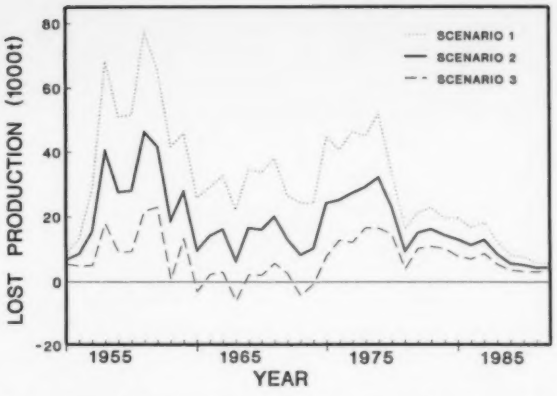

FIG. 3. Potential lost production of pink, chum, and sockeye salmon resulting from Japanese high-seas mothership and land-based driftnet fishing. Lost production varies depending on the mortality schedule chosen for each species, as depicted by scenarios 1, 2, and 3 (description in text).

Marine Survival Trends

We selected marine survival data for Japanese chum hatcheries to compare with the Aleutian Low Pressure Index because the hatchery data were available, the quality was good, and the total fishery represents a large percentage of the recent all-nation salmon catch. Data for Japanese chum hatcheries were from (M. Kaeriyama[1]). The Japanese data do not include estimates of lost production resulting from high-seas interceptions.

Results

Estimates of Lost Salmon Production Resulting from the Japanese High-Seas Salmon Driftnet Fishery

The range of estimates of lost salmon production, calculated using the three scenarios of salmon mortality, was greatest from the mid-1950s to the mid-1970s, coinciding with the peak of the high-seas fishery (Fig. 3). As high-seas fishing effort declined into the 1980s, the estimates of lost production decreased. Scenarios 1 and 3 represent the possible extreme estimates of lost production and were not used to adjust catches. Because scenario 2 represented an average amount of mortality, it was used to estimate lost production. In scenario 2, lost production ranged from approximately 4000 to 45 000 t over the time series. Lost production peaked in the 1950s at about 45 000 t, declined in the 1960s to an average of almost 15 000 t, increased to almost 25 000 t in the early 1970s, and then declined abruptly from 1976 to about 10 000 t in 1978 and gradually declined to about 4000 t by 1989 (Fig. 3).

[1]Paper presented at the International Symposium on Biological Interactions of Enhanced and Wild Salmonids, June 1991, Nanaimo, B.C. (unpublished).

Ricker (1976) reported that gains from eliminating Japanese high-seas fishing would be 76% of the weight of fish caught in the high-seas fishery for North American sockeye and 86% for Asian sockeye. Our estimates of lost production are smaller than previous estimates (Ricker 1964), mainly because we did not incorporate noncatch mortality (or "dropout") into our calculations. Other differences resulted because Ricker (1964) based growth data on back-calculations using interannuli distance on scales whereas we use mean weight per fish of high-seas and coastal catches. Ricker's calculations show an overall production loss to coastal catches equivalent to approximately 60% of the high-seas catch for all species combined whereas our calculations show an overall loss of about 6%. We used our estimate of lost production, but if we used the higher estimates, the catch trends would be more similar to the index of climate trends.

Catch Trends

Combined all-nation catches of pink, chum, and sockeye salmon (Fig. 4A) averaged 673 100 t from the mid-1920s to the early 1940s (1926–43). Maximum catches occurred in the late 1930s with an historic high catch of 837 400 t in 1939. A period of low catch occurred from the mid-1940s until the mid-1970s, except for a small increase around the mid-1950s (average from 1944 to 1975 was 374 100 t). The lowest total salmon catch of 275 600 t occurred in 1974. In the most recent years, production increased. The 1985 all-nation catch was 719 641 t, the highest in five decades and 86% of the historic high catch in 1939 (Fig. 4A). When the lost production calculated from scenario 2 (Fig. 4A) was added to the all-nation catches of pink, chum, and sockeye salmon, the increase in the mid-1950s was accentuated, but the trend in catch was basically unchanged.

The patterns of the all-nation catch for each of the three species were also similar (Fig. 4C–4H). All LOWESS trends (Fig. 4A, 4C, 4E, 4G) identify high catches from 1925 to the mid- to late 1930s, a decline to the mid-1970s, and then an increase to the end of the data series in 1989. The trends for chum and sockeye salmon indicate that catches in the mid- to late 1980s were equal to or exceeded historic catches in the time series whereas the trends for pink salmon indicated that the catch in the 1980s had not reached previous levels at the beginning of the time series.

The similarity in trends is even more apparent from the cumulative sum analysis (Fig. 4B, 4D, 4F, 4H). Catches of pink, chum, and sockeye salmon were above the long-term mean from 1925 to about 1942, followed by a trend of catches below the long-term mean until 1977 when the trend changed. At this time, chum and sockeye catches increased relative to the long-term mean, while pink catches remained at the long-term mean. The cumulative sum analysis shows a slight change in trends for pink and chum salmon beginning in 1953 and ending from 1956 to 1958. Small differences in the timing of changes in the trends occurred, but the pattern is amazingly similar considering the different management practices in salmon fisheries in the four countries (Shepard et al. 1985) over such a long time series.

The trends in total catches for individual species for each country (Fig. 5) were similar to the general trend in catches observed for combined all-nation and combined individual species catches, but were not as distinct. Salmon catch trends for the United States (mainly Alaska) for pink, chum, sockeye (Fig. 5), and total all-species catch were virtually identical to the trends for all-nation, all-species catches.

Total Japanese catches were high until the early 1940s but were significantly reduced during World War II and for several years afterward (Fig. 5). Catches recovered by the mid-1950s and they remained relatively stable until the mid-1980s. Catches of chum salmon followed the pattern of total catches, except that catches increased dramatically in the mid-1970s in association with the development of a major hatchery program (Kaeriyama 1989). Because the Japanese high-seas fishery catches some chum salmon produced in Russia and the United States, the reported catch only approximates Japanese chum salmon production. Catches of pink salmon oscillated on a downward trend, beginning in the early 1960s. Declining Japanese catches of pink salmon from the mid-1970s mostly reflect reductions in the high-seas interceptions. Japan does not produce anadromous stocks of sockeye salmon; therefore the catch trends of sockeye are not trends of Japanese production, but trends in the interception fisheries.

Russian salmon catches (Fig. 5) appear to have declined slightly during World War II but returned to previous levels until the mid-1950s. Average catches from 1925 to 1933 and from 1941 to 1955 were about equal. Total catches did not increase in the late 1970s and early 1980s, but increased in the late 1980s. Unpublished catch data for 1991 indicate the total Russian catch could be about 250 000 t, the second highest catch in the history of their Pacific salmon fishery since 1925. Chum and pink salmon catches followed the general pattern of total catches, except there were increases in pink salmon catches in the late 1970s. Sockeye salmon catches increased in the early 1920s, declined by the mid-1930s, and remained at low levels until the mid-1980s when there was some indication that catches were increasing.

Canadian catches (Fig. 5) were the lowest of the four countries and catch trends were more variable. Total catches (Fig. 5) were high from 1925 to the early 1940s, were slightly lower up to the early 1950s, increased briefly to the mid-1950s, and remained relatively stable until the early 1980s. The pattern of pink salmon catches was quite variable, but does show a trend to increased catches in the late 1970s. Average chum salmon catches were relatively stable from 1928 to the mid-1950s when the average catch declined. Average sockeye salmon catches did not change until the mid-1950s when there was a small increase; average catches were lower from the late 1950s until the mid-1960s. Average catches increased from the mid-1960s until the late 1970s and again beginning in the early 1980s.

The problems of interceptions that exist when national catch trends are examined can be minimized by looking at catch trends for Asia and North America after correcting for interceptions. The changes in the reported catches resulting from high-seas salmon interceptions and for lost North American production were small relative to the total catch (Table 3). The LOWESS trends (Fig. 6) show that catches in Asia and North America started to decline almost the same time, but the decline was more rapid in Asia. Only the Asian catches showed the small increase in catch in the 1950s. The increase in catch in the late 1970s was much more abrupt in North America (Fig. 6B) than in Asia (Fig. 6A).

Aleutian Low Pressure Index

The Aleutian Low Pressure Index for the years 1900–89 (Table 4; Fig. 7) indicated that sea level pressure was high (low index represents high pressures or less intense lows) over a vast area of the northern North Pacific Ocean at the turn of the century, but the Aleutian Lows intensified steadily until the late

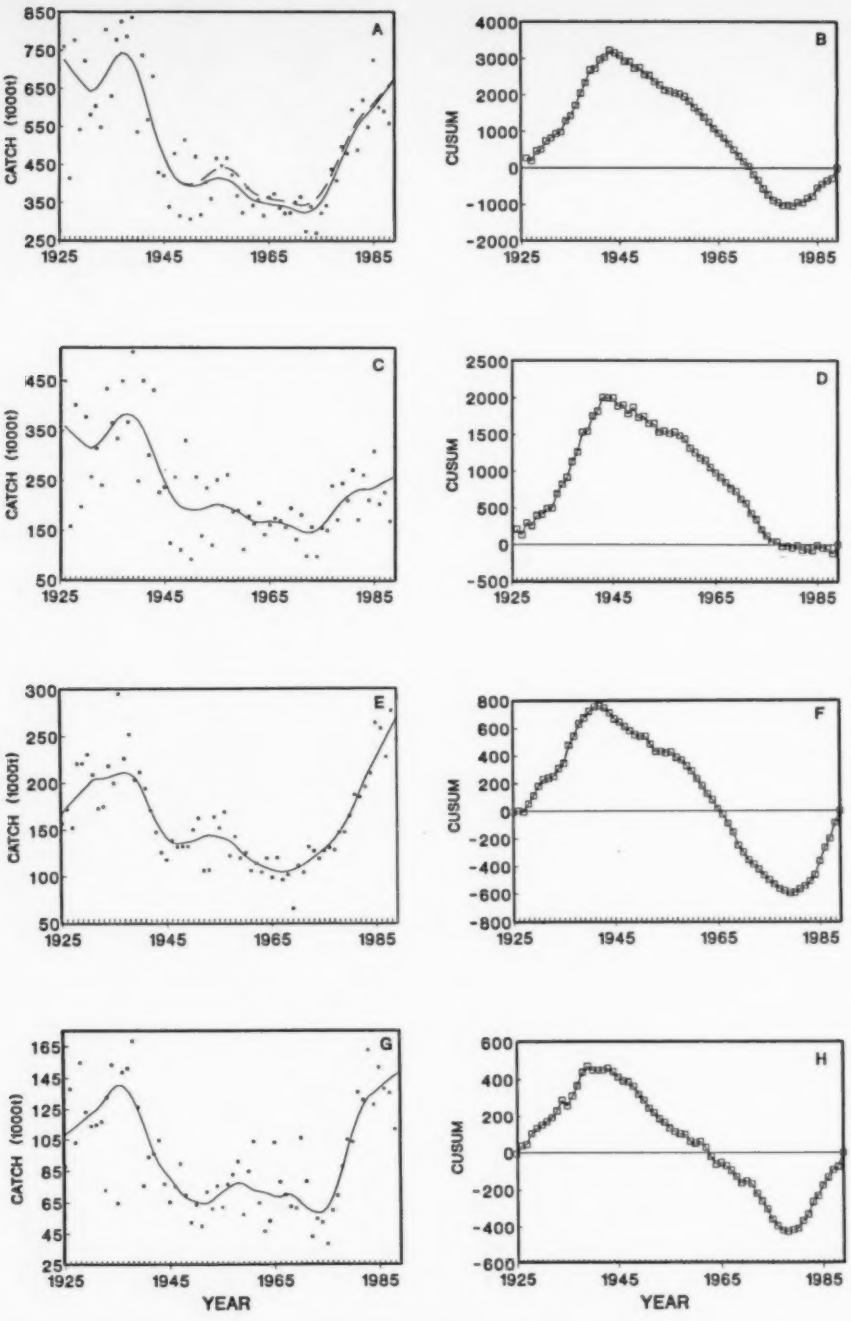

FIG. 4. Trends in catches of (A and B) combined all-nation catches of pink, chum, and sockeye salmon and all-nation catches of each species ((C and D) pink, (E and F) chum, and (G and H) sockeye)). The solid squares in the left-hand panels are the mean annual catches, and the solid line is the LOWESS smoother with a band width, f, of 0.20. The open squares in the right-hand panels are the cumulative sum values for the mean annual catches. The broken line in Fig. 4A indicates the potential increase in production if there was no high-seas salmon driftnet fishery. The cumulative sum analysis (Fig. 4B, 4D, 4F, 4H) shows changes in trends of catches relative to the long-term mean (line at 0). "Increasing" trends represent catches that average higher than the long-term mean; "decreasing" trends represent catches that are lower than the long-term mean.

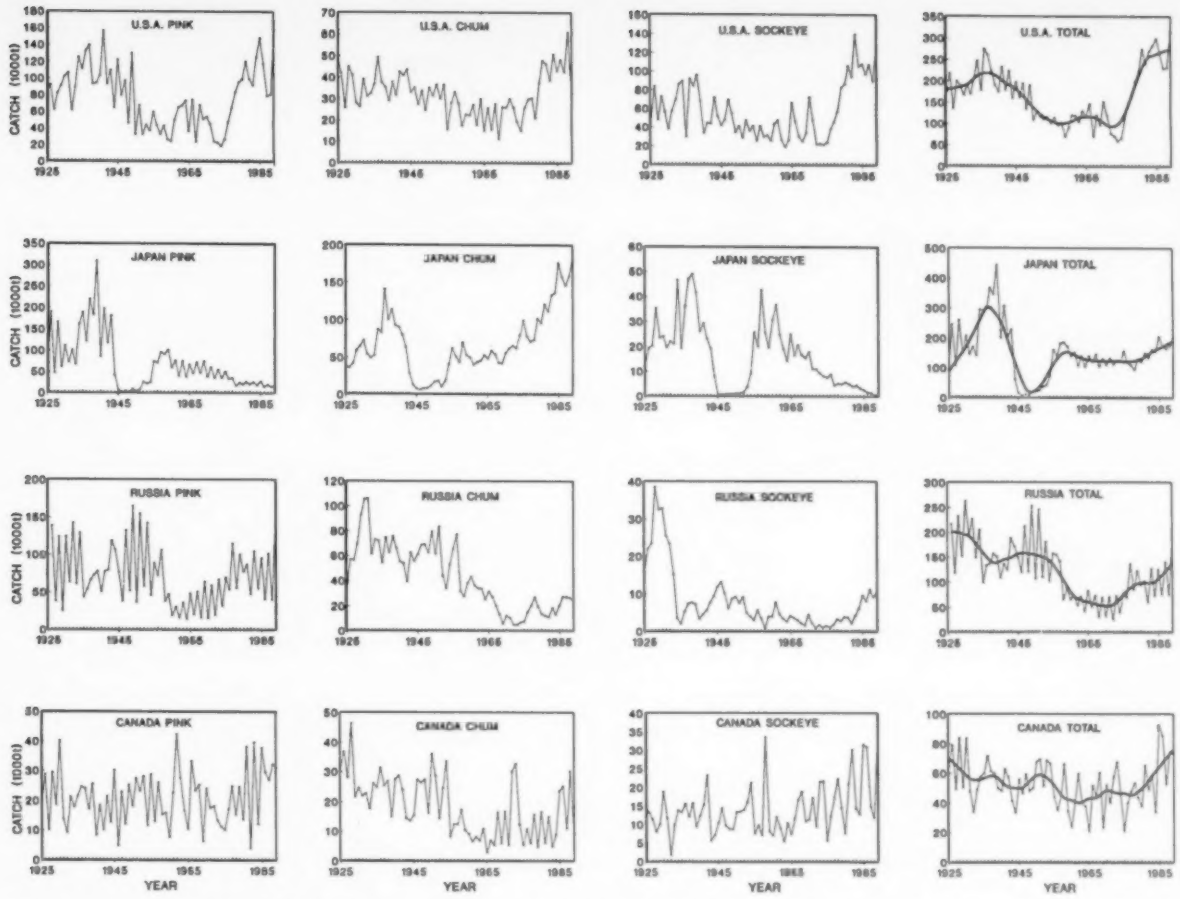

FIG. 5. Catches of pink, chum, and sockeye salmon and the total catch of the three species for United States, Japan, Russia, and Canada. The total catch for each country is fitted with a LOWESS smoother with a band width, f, of 0.20.

1930s (high index represents low pressures or intense lows). From about 1940 to 1950 the index dropped, indicating a change to higher pressures. The period from 1950 to 1970 was characterized by high pressure or weak lows except for a small period of intense lows in the late 1950s and early 1960s. Beginning in the early 1970s, the intensity of the lows again increased, reaching the most intense levels in the time series in the 1980s. In the late 1980s, there was a weakening of the Aleutian Low, but it is too early to determine if the trend has changed.

The pattern of the smoothed Aleutian Low Pressure Index (Fig. 7) corresponded closely to the trend in combined all-nation salmon catches. The declines in catches from the late 1930s to the late 1940s correspond to a sharp decline in the Aleutian Low Pressure Index. Similarly, the increases in catches beginning in the mid-1970s followed an increase in the index. There was a small fluctuation in the salmon production index in the early 1950s when the Aleutian Low Pressure Index changed, but catch trends declined again in the late 1950s (Fig. 7). There was no significant correlation observed when we used linear regression analysis to compare the annual Aleutian Low Pressure Index and the annual North Pacific Ocean Salmon Production or when we compared the Aleutian Low Pressure Index with catches lagged 1 yr for pink salmon, 2 yr for sockeye salmon, and 3 yr for chum salmon.

The pattern of the smoothed sea level pressure at Darwin was very similar to the trend in the Aleutian Low Pressure Index after the mid-1920s (Fig. 8). After the mid-1920s, there were increasing trends in both areas up to about 1940, from the early 1950s to the early 1960s and from the early 1970s until the late 1980s. Decreasing trends occurred from about 1940 to the early 1950s and from the early 1960s to the early 1970s. The similarity in the patterns indicates that sea level pressure trends in the North Pacific were closely related to trends in the tropical South Pacific Ocean but were opposite in phase. The trends at Darwin were actual sea level pressures whereas the Aleutian Low Pressure Index is an index of the area of low pressures. The trends of increasing sea level pressures at Darwin, therefore, were associated with trends of increasing size of the area of low pressure in the North Pacific, i.e., with decreasing sea level pressure. This indicates that the pressure trends in the North Pacific Ocean are similar but opposite in phase to the trends in the tropical South Pacific Ocean. We show the period December–May in Fig. 8B; the trend for the period June–November is not shown, but was almost identical to the December–May period.

TABLE 3. Total catches (t) of pink, chum, and sockeye salmon in Asia and North America and catches corrected for interceptions from 1956 to 1975 resulting from Japanese high-seas salmon driftnet fishery. Catch data were obtained from INPFC (1977–89, 1979) and interception data from Fredin (1980).

Actual catch			Actual catch			Corrected catch	
Year	Asia	North America	Year	Asia	North America	Asia	North America
1925	—	239 600	1956	298 200	148 000	291 386	155 327
1926	462 808	296 700	1957	324 900	141 400	311 157	154 048
1927	227 958	185 600	1958	254 500	167 700	253 695	168 548
1928	493 016	283 700	1959	263 600	104 100	262 244	105 456
1929	301 564	240 000	1960	208 400	113 700	199 425	121 259
1930	470 401	251 900	1961	228 000	160 900	211 894	173 536
1931	342 871	238 100	1962	164 500	177 900	162 286	179 887
1932	399 418	204 000	1963	213 100	143 800	210 870	145 884
1933	291 688	256 000	1964	148 200	166 900	145 731	169 475
1934	500 335	304 700	1965	224 600	138 600	211 091	149 919
1935	391 288	238 100	1966	175 000	197 900	171 307	201 668
1936	432 936	345 700	1967	220 900	115 000	218 899	117 052
1937	503 428	323 200	1968	140 600	180 500	136 928	184 597
1938	503 291	284 200	1969	203 100	119 000	199 301	122 909
1939	595 819	241 600	1970	145 900	203 800	136 497	213 783
1940	311 884	223 900	1971	205 100	159 400	202 025	163 061
1941	442 130	295 500	1972	139 600	133 900	137 415	136 818
1942	330 211	237 400	1973	200 700	138 000	198 881	140 363
1943	417 978	264 400	1974	164 500	104 700	162 290	107 649
1944	235 082	193 700	1975	235 400	86 400	233 697	88 442
1945	166 480	253 600	1976	190 910	150 500	—	—
1946	128 890	209 500	1977	248 020	188 796	—	—
1947	225 040	253 400	1978	182 870	224 351	—	—
1948	130 590	183 400	1979	253 870	243 231	—	—
1949	274 190	240 000	1980	218 140	260 609	—	—
1950	129 130	176 600	1981	254 500	339 427	—	—
1951	272 580	197 400	1982	198 040	289 538	—	—
1952	149 500	168 200	1983	290 520	328 882	—	—
1953	224 900	178 800	1984	233 090	315 698	—	—
1954	178 200	181 600	1985	332 060	392 320	—	—
1955	320 100	145 000	1986	251 500	348 600	—	—
			1987	307 300	282 300	—	—
			1988	252 100	306 100	—	—
			1989	387 100	388 100	—	—

The Nothern Hemisphere surface air temperature anomalies (data from Hansen and Lebedeff 1987) smoothed using a LOWESS band width, *f*, of 0.20 produced a trend that was also similar to the Aleutian Low Pressure Index (Fig. 8). Temperature anomalies increased just after the turn of the century to about 1940, then decreased until about 1970, and then increased up to the late 1980s. The temperature time series shows the small change in trends in the 1950s that was present in the other indices.

The pattern of marine survival of Japanese hatchery-reared chum salmon, the total catch of chum salmon from the three other salmon producing countries, and the Aleutian Low Pressure Index show similar trends (Fig. 9). The period of increasing marine survival of hatchery-reared salmon was closely associated with an increasing catch of wild chum salmon by the other three Pacific salmon producing countries.

Discussion

The LOWESS and cumulative sum analyses show that similar catch trends exist for the combined all-nation catches of pink, chum, and sockeye salmon and the combined all-nation catches for each species. The changes in these trends occurred at almost the same time throughout the northern North Pacific Ocean. When catch trends were examined by country, the trends persisted, although there was more variation, especially when the catches were relatively small (e.g., sockeye in Russia). The pattern of catches for all species in North America was similar to the pattern of catches in Asia, except that Asia catches did not increase as quickly in the late 1970s and 1980s because of low Russian catches. However, in 1991, Russian catches were the second highest in the time series, indicating that the trends in Asian salmon production were similar to North American trends.

The strong similarity of the pattern of pink, chum, and sockeye salmon catches indicates that common events affect the production of salmon in the North Pacific Ocean. These common events influence catch over a vast area despite high fishing effort, different fleet dynamics, different gear, and different management policies. The Aleutian Low is a large-scale climate system that affects vast areas of the northern North Pacific Ocean. Our index of the intensity of this system closely matched the trend in all-nation salmon production.

There is considerable variation among the life histories of pink, chum, and sockeye salmon as well as some variation in life history within each species. Climate variation, therefore, can influence survival over a number of years, making it difficult to identify correlations between salmon survival and envi-

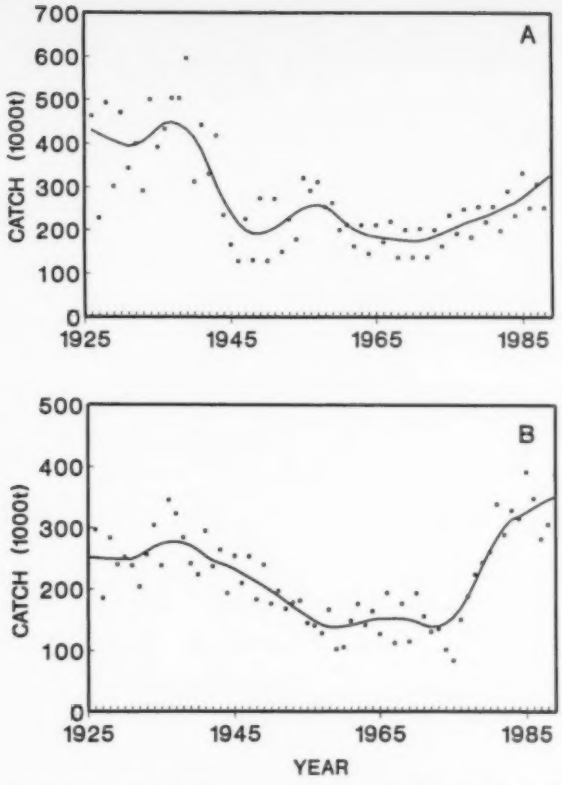

FIG. 6. Trends in catches of the combined (A) Asian and (B) North American catches of pink, chum, and sockeye salmon. The solid squares in Fig. 6B are the mean annual catches adjusted for high-seas interceptions (see text), and the solid line in both panels is the LOWESS smoother with a band width, *f*, of 0.20.

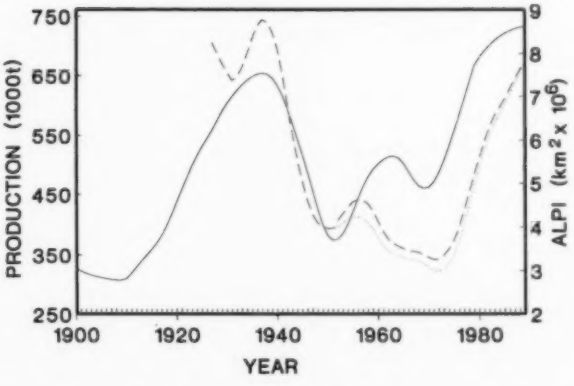

FIG. 7. Comparison of the combined all-nation catch (production) of pink, chum, and sockeye salmon, including lost production resulting from the high-seas salmon driftnet fishery (broken line) and the smoothed Aleutian Low Pressure Index (solid line), produced using a LOWESS smoother with a band width, *f*, of 0.20, from the annual values in Table 4. The dotted line represents the smoothed all-nation catch that is not adjusted for lost production resulting from high-seas interceptions.

TABLE 4. Annual Aleutian Low Pressure Index (km²) for winter and spring for the years 1900–89. Data for December–May.

Year	Aleutian Low	Year	Aleutian Low
1900	6 660 225	1945	No data
1901	1 871 775	1946	8 732 025
1902	6 323 400	1947	2 132 775
1903	0	1948	2 371 275
1904	8 775	1949	3 011 625
1905	4 000 050	1950	1 685 250
1906	2 589 750	1951	3 047 850
1907	0	1952	1 984 050
1908	4 484 475	1953	9 560 475
1909	1 938 375	1954	3 785 175
1910	0	1955	2 679 525
1911	0	1956	255 600
1912	7 933 275	1957	3 125 025
1913	3 875 850	1958	10 322 550
1914	8 973 675	1959	2 909 475
1915	1 356 975	1960	6 738 975
1916	0	1961	10 583 325
1917	3 529 575	1962	3 803 850
1918	5 504 625	1963	10 542 825
1919	7 616 250	1964	7 713 000
1920	27 000	1965	2 834 100
1921	5 858 100	1966	4 033 575
1922	143 550	1967	6 862 950
1923	3 840 750	1968	4 968 225
1924	6 446 475	1969	1 274 625
1925	5 290 650	1970	10 449 900
1926	14 023 800	1971	129 825
1927	8 216 550	1972	1 800
1928	7 562 475	1973	2 335 050
1929	8 777 475	1974	5 881 725
1930	1 669 950	1975	5 673 825
1931	11 310 300	1976	6 165 900
1932	1 731 600	1977	11 306 025
1933	3 368 475	1978	10 462 950
1934	10 867 275	1979	4 457 925
1935	6 318 000	1980	11 967 525
1936	11 192 625	1981	13 409 775
1937	1 024 200	1982	2 475
1938	7 877 700	1983	13 959 000
1939	7 605 450	1984	6 321 150
1940	15 435 225	1985	5 001 075
1941	13 852 800	1986	12 646 125
1942	9 082 575	1987	10 624 050
1943	2 581 875	1988	8 901 225
1944	8 431 875	1989	2 632 050

ronmental variation. The low correlations between the annual Aleutian Low Pressure Index and annual salmon production, either lagged or unlagged, indicate that the annual relationship between all-nation salmon production and the Aleutian Low Pressure Index is highly variable either because climate-induced productivity changes occur over a number of years or there is much interannual variation not explained by the general climate index or both. We did examine correlations based on 5-, 7-, and 11-yr running averages of the Aleutian Low Pressure Index and annual all-nation salmon catches of all species. The correlations were considerably higher; however, analyzing data in this manner creates new sets of problems that we felt were better left for another paper.

It is possible that factors other than the environment affected the production trends. For example, overfishing during the early high-production period (1925–40) may have reduced production and resulted in the observed long period of low catches

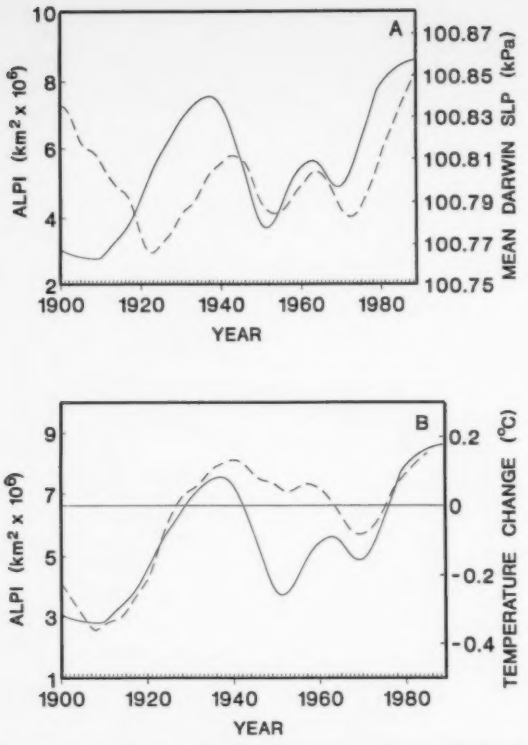

FIG. 8. (A) Aleutian Low Pressure Index (solid line) compared with sea level pressure at Darwin (broken line), smoothed using a LOWESS smoother with a band width, *f*, of 0.20. (B) Northern Hemisphere mean surface air temperature anomalies smoothed using a LOWESS smoother with a band width, *f*, of 0.20 (broken line) compared with the Aleutian Low Pressure Index (solid line).

from 1945 to 1975. It is difficult to compare returns per spawner for the stocks involved and for the time series we used. However, when we examined the Japanese hatchery data, there was evidence that indicated that production of salmon from the early 1970s to the present was associated with improved marine survival. Higher pink salmon survival of both hatchery and wild stocks in Alaska during this same period was associated with higher ocean temperatures during the early marine period (Willette and Cooney 1991 and unpublished data; D.M. Eggers et al.[2]). However, the most convincing argument that trends are not the result of management actions in response to overfishing is the consistent similarity among trends. It is unlikely that overfishing periods would be similar among the four countries for all three species and that at about the same time, each management agency in each country would initiate programs of rebuilding that resulted in an almost simultaneous improvement in abundance.

Artifical rearing or enhancement activities may have contributed to the increases in production starting in the mid-1970s. Our data series ends in 1989, so brood years (year of spawning) of sockeye and chum salmon in the 1989 catch occurred in the mid-1980s (most sockeye salmon return to spawn at age 4 and

[2]Paper presented at the International Symposium on Biological Interactions of Enhanced and Wild Salmonids, June 1991, Nanaimo, B.C. (unpublished).

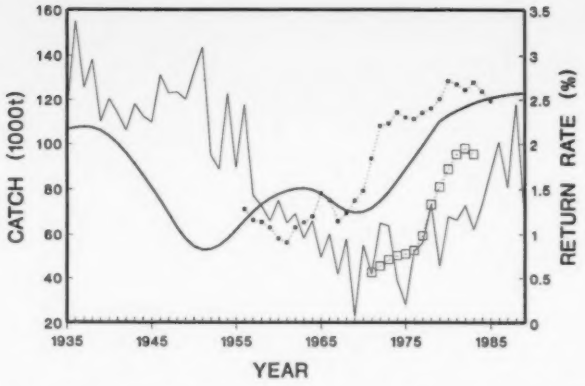

FIG. 9. Total annual North Pacific Ocean catch of chum salmon (not smoothed) by Canada, the United States, and Russia (solid line) compared with Japanese hatchery-reared chum survival rates for Hokkaido (solid squares) and Honshu (open squares). The survival rates are shown as 5-yr means. The heavy solid line is the smoothed Aleutian Low Pressure Index from Fig. 7.

5, and most chum salmon return to spawn at ages 4–6) and pink salmon in the late 1980s (almost all pink salmon return after 18 mo at sea).

In Japan, chum salmon production is now exclusively from hatcheries. From 1972 to 1982, releases of chum salmon fry from hatcheries increased from 800 million to 2 billion fry (M. Keriyama[1]). Marine survival of these fry increased from 1 to 2% in about 1966, apparently because of changes in hatchery practices (Kaeriyama 1989). In 1976, 1977, and 1981, the marine survival was exceptionally high at 3% (M. Kaeriyama[1]). Pink salmon are also produced in Japanese hatcheries, but production is relatively small (138 million fry in 1989 or about 6400 t of returning adults using an average marine survival of 1.5% and an average weight in the coastal fishery of 1.6 kg). M. Kaeriyama[1] noted that the highest marine survival of hatchery-reared pink salmon of 3.8% occurred for pink salmon going to sea in 1977. Our comparison of catches of chum salmon by Canada, the United States, and Russia (Fig. 9) with changes in the marine survival of chum reared in Japanese hatcheries shows a similar pattern between climate change and marine survival. There is no question that the increasing catches of chum salmon in Japan in the 1970s were a result of increasing hatchery production, but the increase in marine survival in the late 1970s and early 1980s indicated that there were changes in the marine environment that contributed to this improved survival.

Salmon hatcheries in Russia produced about 480 million pink salmon fry and 320 million chum salmon fry in the 1970s and about 25% fewer fry in the 1980s (V.M. Chupakhin et al.[3]). From 1976 to 1990, catches of chum salmon increased (V.M. Chupakhin et al.[3]) despite reductions in releases from hatcheries, indicating that survival of hatchery fish in the marine environment improved. Using an average marine survival estimate of 1.5% for pink salmon and 1% for chum salmon, and an average weight of 1.6 kg for pink and 3.6 kg for chum, we estimated that hatchery production in Russia could account for an annual production of about 9000 t of chum salmon and 9000 t of pink salmon in the mid-1980s. This production would

[3]Paper presented at the International Symposium on Biological Interactions of Enhanced and Wild Salmonids, June 1991, Nanaimo, B.C. (unpublished).

be a significant amount of the chum salmon catch, if harvest rates were high (70–90%), but not of the pink salmon catch. Thus, the addition of hatchery-produced pink salmon would not explain the increased catches that began in the late 1970s.

Alaska is the major producer of salmon caught in the United States. In the central Gulf of Alaska, hatchery-produced pink salmon contributed about 5% of the total pink salmon biomass in 1980 and 27% of the total biomass in 1985 (D.M. Eggers et al.[2]). Thus, hatchery production contributed to the increases in catch from the early to mid-1980s. However, production from the wild runs also increased three to four times from the mid-1970s to the mid-1980s (D.M. Eggers et al.[2]). In the mid-1980s, approximately 3300 t of adult chum salmon and 2100 t of adult sockeye salmon were produced from Alaskan fisheries enhancement programs (estimates made from total releases in 1983, assuming a marine survival rate of 1% for chum and sockeye salmon and an average weight of chum salmon of 3.6 kg and sockeye of 3.2 kg). The increases in catch of both chum and sockeye salmon in the late 1970s were so large that the contribution from hatcheries and enhancement would represent only a small amount of the increased production.

In Canada, pink salmon hatchery production was relatively small, contributing less than 500 t up to the mid-1980s. The average increases in total catch from the mid-1970s to mid-1980s would therefore not be a direct result of enhancement. Sockeye salmon enhancement from 1975 to 1980 resulted in an annual increase in catch of about 1500 t by 1980 and 3000 t by 1985 (Salmon Enhancement Program, Vancouver, B.C., Canada, unpublished data). This would be an important contribution to the increase in catch but would not account for the average increase in catch from 1975 to 1985. Hatchery production of chum salmon in the Canadian commercial catch increased from about 200 t in the mid-1970s to about 7000 t in 1985. Chum salmon catches that increased in the mid-1980s appear to be associated with the increased catch of enhanced fish. The Canadian contribution of chum salmon to the total all-nation catch, however, was only about 8% during this period.

It is difficult to separate the increases in salmon abundance resulting from improved ocean survival from increases in abundance that could result from improvements in productivity in the freshwater environment. Because the increases in salmon survival in the late 1970s occurred at the same time that year classes of a number of other commercially important marine fishes had exceptionally high marine survival (Beamish 1993), it appears that there was a general increase in production of commercially important marine fish species beginning in the late 1970s. The reason for the exceptionally strong year classes of marine fishes is believed to be the large increases in food for larval fishes that occurred in the late 1970s (Brodeur and Ware 1992; McFarlane and Beamish 1992; Beamish 1993). We reexamined the relationship reported by McFarlane and Beamish (1992) using updated annual Aleutian Low Pressure Index data for the 6-mo period December–May and the unweighted mean monthly (March–May) copepod abundance estimates for ocean station P (50°N, 145°W; Fig. 2). We used a standard linear regression because the residuals were not significantly autocorrelated at the 5% level. We found that there was a significant relationship between copepod abundance and the Aleutian Low Pressure Index (Fig. 10; $r = 0.50$, $p < 0.05$). Although this relationship was not as strong as estimated by McFarlane and Beamish (1992), it shows that there is a close relationship between the average intensity of the Aleutian Low and the average production of copepods. The average annual production of copepods from 1965 to 1975 was 86.6/m^3 compared to 129.8/m^3 from 1976 to 1980, a 1.5 times increase in abundance. If we used our calculated relationship to estimate copepod abundance from 1981 to 1989, the extrapolated estimates would indicate a 5–6 times increase in abundance if the relationship continues outside the range of the data. We believe that the differences in average copepod production between the two periods are evidence that there was a large increase in the abundance of copepods beginning in 1976 that was associated with the change towards more intense Aleutian Lows (Fig. 7). Because copepods are a principle food for many marine species, including salmon and prey of salmon, and because copepods are the dominant species in the zooplankton (McFarlane and Beamish 1992), we believe that increases in salmon production are closely related with the increases in zooplankton production in the marine environment.

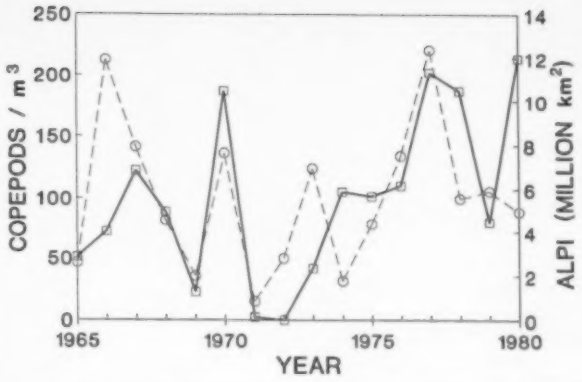

FIG. 10. Relationship between copepod abundance at Ocean Station P (broken line) and the Aleutian Low Pressure Index (solid line). Data not smoothed.

If climate over the northern North Pacific Ocean affects survival of Pacific salmon and results in long-term trends in production, there should be evidence of density-dependent growth. Such a carrying-capacity effect would likely be related to food availability on a large geographic scale. Ishida et al. (1993) showed significant increases in mean age of Japanese and Russian chum salmon stocks and decreases in mean body weight. These changes occurred during the period of increasing abundance in the 1970s and 1980s. Rogers (1980) and Peterman (1984) have also identified density-dependent growth in North American stocks of sockeye salmon. Thus, although the evidence is limited, there are examples of density-dependent growth during the 1980s. It is interesting that the trend towards decreasing size occurred during a period when food apparently was increasing. A relationship between decreasing fish size and increasing ocean productivity trends would also be expected to result in increases in the trends of fish size during periods of decreasing trends in productivity.

We concluded that the trends in salmon production from 1925 to 1989 were not primarily a result of fishing effort, management actions, or artificial rearing, but rather that the trends in abundance were strongly linked to the environment. The robustness of the general trend in the three species for all countries over such a vast geographical area, the continuous rather than precipitous change in abundance, and the close association of the trends with trends in the Aleutian Low suggest that this

vast low pressure system is associated with the changes in salmon abundance. The mechanism involved could result from increased upwelling in the ocean under the centre of the Aleutian Low (Reid 1962; Thompson 1981) resulting in increased productivity (Venrick et al. 1987; Brodeur and Ware 1992; McFarlane and Beamish 1992). The resulting horizontal divergence (Reid 1962) would transport plankton and nutrients along the edge of the coast of North America. Off the Pacific coast of Asia, it is known that north–south shifts in the Aleutian Low affect winter sea surface temperatures in the western North Pacific Ocean off the coast of Japan and the strength of the East Asian Winter Monsoon over Japan (Hanawa et al. 1989). A northern shift in the Aleutian Low produces warmer winters and weaker East Asian Winter Monsoons, while a southern shift in the Aleutian Low produces colder winter sea surface temperatures and stronger East Asian Winter Monsoons (Hanawa et al. 1989), confirming that the strength and position of the Aleutian Low affect ocean conditions along the Asian coast. We are not aware of any studies that relate changes in the intensity of the Aleutian Low with plankton production along the Asian coast; however, the relationship between the position of the Aleutian Low and large-scale effects on sea surface temperatures in the western North Pacific Ocean shown by Hanawa et al. (1989) suggests that the intensification of the Aleutian Low could effect the productivity of vast areas of the northern North Pacific Ocean.

The smoothed trend in the Aleutian Low Pressure Index was similar to the smoothed trend in sea surface pressure at Darwin after the mid-1920s (Fig. 8A) and to the trend in the Northern Hemisphere mean sea surface temperature change (Hansen and Lebedeff 1987) (Fig. 8B), indicating an association among the climate indicies. The use of the Hansen and Lebedeff (1987) temperature series shows trends that are similar to the Aleutian Low Pressure Index, but it over simplifies the pattern of sea surface temperature trends. McLain (1984) and Trenberth (1990) both showed that the pattern of sea surface temperature anomalies tends to be opposite along the west coast of Canada and the United States compared with the open ocean. Trenberth and Paolino (1981) and Niebauer (1988) suggested that climate changes reflected by differences in sea surface pressure between Darwin and Tahiti (the Southern Oscillation Index) are linked to climate anomalies in the Northern Hemisphere and Bering Sea by the Aleutian Low pressure system. The parallel smoothed trends of the sea surface pressure at Darwin and the Aleutian Low Pressure Index are opposite in phase, indicating that an oscillation in pressure trends occurs between the tropical South Pacific Ocean and North Pacific Ocean and are additional evidence that a linkage exists between the climate trends in these two areas.

Climate events over the tropical South Pacific Ocean are associated with sea surface temperatures in the northern North Pacific Ocean and the Bering Sea (Iwasaka et al. 1987; Yasunari 1987a, 1987b; Hanawa et al. 1988; Niebauer 1988). Sea surface temperatures, therefore, as well as sea surface pressures are linked between the South and the North Pacific through the atmospheric circulation and the intensification of the Aleutian Low (Namias 1959, 1963, 1969; Davis 1976, 1978; Blackmon et al. 1983; Lazante 1984; Wallace et al. 1990; Alexander 1992a, 1992b). We point out the similarity in the trends in these indices of sea surface pressure, land temperatures, and the associations between climate events in the South and North Pacific to show that salmon production trends may be associated with climate variables other than the Aleutian Low Pressure Index and with climate events in areas other than the North Pacific Ocean.

The Aleutian Low Pressure Index was the lowest in the time series at the turn of the century. Trenberth and Paolino (1980) showed that the sea level pressure data were most reliable after 1924; however, if the index is valid for the early part of the time series and if it followed the same relationship with salmon abundance up to 1925 as it did after 1925, then the all-nation salmon abundance must have been low just after the turn of the century. It is difficult to confirm if this reduced abundance occurred because modern salmon fisheries were just developing and catch records, when they exist, are not necessarily reliable indicators of total abundance. In Japan, Kaeriyama (1989) reported that the wild fishery for chum salmon in Hokkaido was in an early development phase beginning in 1870 and reaching a maximum of 11 million individuals in 1889. Catches declined sharply over the next 20 yr, as a result of declining stock abundance, to a low of 1.2 million fish in 1909. The decline was believed to have occurred because of overfishing and poor hatchery practices. Hatcheries started in 1887 and by 1892 were releasing 16 million fish; thus, it is possible that the hatchery program was affecting wild fish production. Nevertheless, there was a dramatic decline in the catch of the Hokkaido fishery at the turn of the century. However, there was only a small increase in production in the 1920s and 1930s when the Aleutian Low Pressure Index was increasing. The factors affecting catches are not known, but it does appear that production declined at the turn of the century. In Canada and the United States, the early salmon fishery occurred mostly for sockeye salmon in the vicinity of the Fraser River and Bristol Bay. The Fraser River stocks were believed to have declined beginning in 1898 for low-cycle runs and after 1913 for all runs (Gilbert 1918; Rounsefell and Kelez 1938; Ricker 1987). The decline in abundance before 1913 was believed to result from overfishing and after 1913 from a combination of overfishing and the obstruction caused by a rockslide in 1913 (Gilbert 1918; Rounsefell and Kelez 1938; Ricker 1987). In the United States, in Bristol Bay, the sockeye salmon fishery developed rapidly in the late 1890s and by 1913 was about 20 million fish (Minard and Meacham 1987). Catches of about 16 million sockeye salmon were sustained until the late 1930s. There was no decline in catch at the turn of the century, possibly because the fishery was still developing. Thus, it is difficult to unravel the reasons for the trends in catches of salmon at the beginning of the salmon fishery in the late 1800s, but it is also difficult to dismiss the possibility that abundance at this time was low, as might be indicated by the trend in the Aleutian Low Pressure Index for the early part of the time series.

Total all-nation catches show that pink, chum, and sockeye salmon production varied from 837 400 to 275 600 t from 1925 to 1989. Our study indicates that this variation in production is associated with trends in the environment. The pattern of the Aleutian Low Pressure Index indicates that eventually it will become less intense (i.e., a return to higher pressures). In the most recent years, there have been some less intense Aleutian Lows, possibly indicating a weakening trend. If this weakening trend remains and if other factors such as increased ocean temperatures do not change the relationship between the Aleutian Low Pressure Index and Pacific salmon abundance, we would predict that Pacific salmon abundance will begin to decline.

Changes in trends in production, possibly as a result of shifts in the structure of ecosystems, have a direct effect on salmon management. The production trends identified in this study

indicate that it is not appropriate to assume that the factors regulating salmon populations have been more or less constant or have varied randomly over the time series in this study. Furthermore, the trends indicate that the management of salmon should be focused on larger aggregates of stocks as well as individual stocks. Strategies for artificially rearing Pacific salmon to improve catches should also consider the effects of production trends. Hatchery production appears to have followed the trends in wild production in the 1970s and 1980s and may have assisted in the rate of increase of abundance by providing large numbers of smolts at a time of improved marine survival. However, when production trends change, it may not be an appropriate strategy to continue to release large numbers of artificially reared smolts during a period of decreasing marine survival of salmon. It is important to recognize and understand the causes of large-scale, long-term patterns in salmon abundance because declines in marine survival should be managed differently from declines resulting from fishing mortality that is too high.

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Pumping Rates and Projected Filtering Impacts of Juvenile Zebra Mussels (*Dreissena polymorpha*) in Western Lake Erie

Christopher M. Bunt, Hugh J. MacIsaac,[1] and W. Gary Sprules

Department of Zoology, University of Toronto, Erindale College, Mississauga, Ont., Canada L5L 1C6

Bunt, C.M., H.J. MacIsaac, and W.G. Sprules. 1993. Pumping rates and projected filtering impacts of juvenile zebra mussels (*Dreissena polymorpha*) in western Lake Erie. Can. J. Fish. Aquat. Sci. 50: 1017–1022.

Small-bodied (2–11 mm), settled zebra mussels (*Dreissena polymorpha* Pallas) comprise up to 90% of individuals inhabiting reefs in western Lake Erie. We assessed pumping rates of these size classes of *D. polymorpha* by injecting an inert dye into inhalant filtering currents and monitoring exhalant flows using high-resolution videography. Pumping rates ranged between 0.20 and 4.45 $mL \cdot ind^{-1} \cdot h^{-1}$ and increased in relation to mussel shell length. Based on 1990 size–frequency distributions for reefs in western Lake Erie and our pumping rate – shell length regression, small settled *D. polymorpha* were theoretically capable of pumping between 39 and 96% of the water column daily. Small-bodied mussels inhabiting Sunken Chicken Reef were collectively capable of processing between 110 and 400% of the values previously reported for *Daphnia*. Recent changes in water quality in western Lake Erie could be primarily related to zebra mussel filtering activities, including those of small-bodied individuals.

Les individus fixés de petite taille (2–11 mm) composent jusqu'à 90 % des populations de moules zébrées (*Dreissena polymorpha* Pallas) qui habitent les récifs de l'ouest du lac Érié. Nous avons calculé le rythme de pompage de ces classes de taille de *D. polymorpha* en injectant un colorant inerte dans les courants aspirés au filtrage et en contrôlant les courants rejetés par vidéographie à haute résolution. Le volume pompé se situait entre 0,20 et 4,45 $mL \cdot ind^{-1} \cdot h^{-1}$, et augmentait en fonction de la longueur des mollusques. À partir des distributions des fréquences par taille calculées en 1990 sur les récifs de l'ouest du lac Érié et de notre régression volume de pompage – longueur de la coquille, il apparaît que les petites moules zébrées fixées étaient théoriquement capables de pomper chaque jour entre 39 et 96 % de la colonne d'eau. Les moules de petite taille habitant le récif Sunken Chicken étaient collectivement capables de traiter entre 110 et 400 % du volume signalé antérieurement pour *Daphnia*. Les changements récemment observés dans la qualité de l'eau de l'ouest du lac Érié pourraient être avant tout liés aux activités de filtrage des moules zébrées, notamment celles des individus de petite taille.

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Zebra mussels (*Dreissena polymorpha* Pallas) are now a component of the benthos in all five of the North American Great Lakes (Griffiths et al. 1991). One of the primary influences zebra mussels are expected to have on Great Lakes ecosystems is a general grazing-induced reduction in phytoplankton abundance. *Dreissena polymorpha* adults are capable of filtering particles ranging in diameter from 0.7 μm (Sprung and Rose 1988) to 750 μm (Ten Winkel and Davids 1982). Considering the broad size classes of food ingested, and the extraordinary population densities of settled animals — up to 3.41×10^5 $ind \cdot m^{-2}$ in western Lake Erie (MacIsaac et al. 1991) — *D. polymorpha* may well become the primary herbivore in some regions of the Great Lakes, particularly in western Lake Erie and Lake St. Clair.

The western basin of Lake Erie has undergone substantial change during the past 4 yr coincident with the establishment and growth of *D. polymorpha* populations. For example, Leach (1992) reported that mean Secchi disc transparency in the western basin increased by 85% between 1988 and 1989, while chlorophyll *a* concentrations during the same years decreased by 43%. These changes were further accentuated during 1990 (Leach 1992). To assess the role of *D. polymorpha*, if any, in promoting these changes, the size–frequency distributions, length-specific clearance rates, and densities must be known for populations in western Lake Erie.

Clearance rate refers to the volume of water from which a suspension-feeding animal has removed all of the food per unit time. A number of studies have been undertaken to quantify clearance rates of *D. polymorpha* of different body sizes (Kryger and Riisgård 1988, and references cited therein). In these studies, clearance rate was measured indirectly by monitoring the depletion of suspended natural algal foods or food analogues, including clay and graphite particles. Clearance rate is equal to the rate at which water flows through the mantle cavity (i.e., pumping rate) only when particles are strained and retained with 100% efficiency (Coughlan 1969). Jørgensen et al. (1984) reported that *D. polymorpha* retained particles >4 μm with 100% efficiency. To date, however, no measurements of either clearance rate or pumping rate have been made for *D. polymorpha* of ≤6.5 mm shell length. Clearance rates have not been measured for juvenile *D. polymorpha*, presumably due to difficulties in handling small individuals and, more importantly, because each individual would be capable

[1]Present address: Department of Biological Sciences, University of Windsor, Windsor, Ont., Canada N9B 3P4.

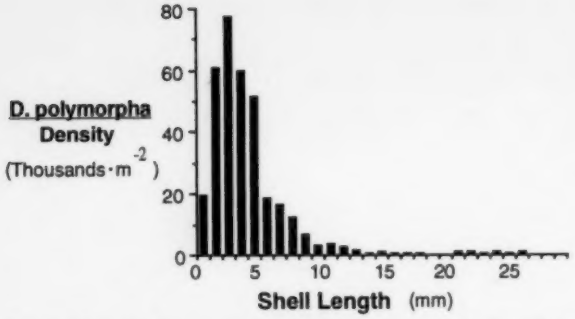

FIG. 1. Mean size–frequency distribution of *D. polymorpha* from eight sites on the Sunken Chicken Island reef in the western basin of Lake Erie during October 1990. Population densities and size distributions were estimated from the collection of representative rock samples from each site (modified from Leach 1992).

of depleting food resources only slightly. Knowledge of clearance and pumping rates is essential, however, to calculations of population filtering impact because a large percentage of some populations, including those in Lake St. Clair (Griffiths et al. 1991) and western Lake Erie (Fig. 1; Leach 1992), are composed of individuals of <10 mm shell length. The purpose of this study is to directly measure the pumping rates of these abundant size classes of *D. polymorpha* (2–12 mm shell length) using a nontoxic dye and high-resolution videography. In addition, we estimate the filtering potential of small (<12 mm) *D. polymorpha* inhabiting three reefs in western Lake Erie.

Methods and Materials

Dreissena polymorpha were gathered near Wheatley, Ontario, in the west-central basin of Lake Erie in mid-June 1991. Animals were maintained in controlled environment chambers at 20°C in aerated aquaria containing glass-fibre-filtered (Whatman 934-AH; retention to 1.5 μm) Erindale Pond water (Erindale College, University of Toronto, Mississauga, Ont.). Batch cultures of the unicellular, motile alga *Cryptomonas* sp. (7 × 14 μm), a preferred food for *D. polymorpha* (Ten Winkel and Davids 1982), were provided every 2 d at a concentration of approximately 9×10^3 cells·mL^{-1} (1.46 μg dry wt·mL^{-1}). Animals were exposed to a 15 h light : 9 h dark photoperiod, which approximated natural conditions at the time of collection. Animals were acclimated to these conditions for at least 10 d prior to experimentation.

Individual mussels, with shell lengths measuring 2–12.5 mm, that appeared to be healthy (i.e., siphons extended) were haphazardly selected during June and July 1991 for experimentation. Shell length was measured to the nearest 0.01 mm with a Fowler ultra-cal II digital caliper. A microcomputer-based caliper measurement system (Sprules et al. 1981) was used to measure the major and minor axes (to the nearest 0.001 cm) of the elliptical exhalant siphon aperture of a pumping individual in a small glass aquarium (5 × 5 × 5 cm). The means of four measurements per axis per individual were used in the determination of pumping rate. In order for the siphon dimensions to remain relatively constant between this measurement process and the dye-injection process, it was necessary to maintain the constancy of the microenvironment around the mussels at all times. The siphon measurement process and the dye-measurement process were conducted in filtered Erindale Pond water at 20°C with a *Cryptomonas* sp. density of approximately 5×10^3 cells·mL^{-1} (0.81 μg·mL^{-1}) to simulate natural food conditions. This food density falls within the range found in western Lake Erie and is apparently below *D. polymorpha*'s incipient limiting concentration (i.e., the food level above which pumping rate declines (Walz 1978)). In addition, care was taken to ensure that shells were fully gaped and siphons fully extended before measurements were made. As such, all mussels were pumping at or near their maximum capacity (Jørgensen et al. 1988). The exhalant plume was made visible by injecting a small quantity of red, unflavoured food colouring (Club House Foods Inc.) near the aperture of the inhalant siphon of a pumping mussel using a borosilicate micropipette with a drawn out tip. On the rare occasion that a test animal responded to the dye, pumping ceased only for a few seconds and was most probably the result of hydromechanical disturbance associated with the micropipette tip. Coughlan and Ansell (1964) reported similarly insignificant responses to a nontoxic dye by their siphonate bivalves.

Exhalant flow velocity was determined by recording the field directly adjacent to the exhalant siphon of a pumping mussel with a high-resolution video system coupled with a time–date generator. This process was performed in a 7-L aquarium with the same food concentration (5×10^3 cells·mL^{-1}) and temperature (20°C) as the siphon measurement process. Mussels were allowed to acclimate for at least 5 min before recording began. At this time, a stopwatch on the time–date generator was started and dye was carefully introduced close enough to the inhalant siphon that it was drawn within the mantle cavity (Fig. 2A). This was best achieved by guiding the micropipette from above rather than from the side of the aquarium. Each trial lasted approximately 5 min, during which it was assumed that the exhalant aperture area remained constant as previously measured (C.M. Bunt, personal observation). A valid plume was one with a clearly discernable leading edge and a linear and laminar flow path over the distance measured (Fig. 2B).

Following repeated dye injections, a reference scale was placed in the field of view of the camera at approximately the same angle as the exhalant plumes to correct for camera magnification and the angle of the exhalant plume travel. The velocity of the exhalant plume was measured over distances from 1.25 to 10 mm depending on animal size. Exhalant flow velocities were transcribed by marking a distance, l, on the video screen and determining the time, t (to the nearest 1/100 s from the time–date generator), for the plume to traverse the distance. From five to nine travel times were averaged per individual for use in the pumping rate formula for animals of each size class.

Pumping rate (PR, millilitres per individual per hour) was calculated as

$$PR = [(\pi \cdot a \cdot b)/4] \times (3600 \cdot l/t)$$

where a and b are the mean minor and major axes, respectively, of the exhalant siphon (centimetres), l = length of exhalant plume travel (centimetres) and t = the time (seconds) required for the plume to travel distance l. Shell lengths, siphon areas, plume velocities, and computed pumping rates for individual mussels are available on request from the authors.

Results and Discussion

Shell length represents an easily measured variable that is correlated with less readily quantifiable aspects of *D. polymorpha* morphology and feeding biology. For example,

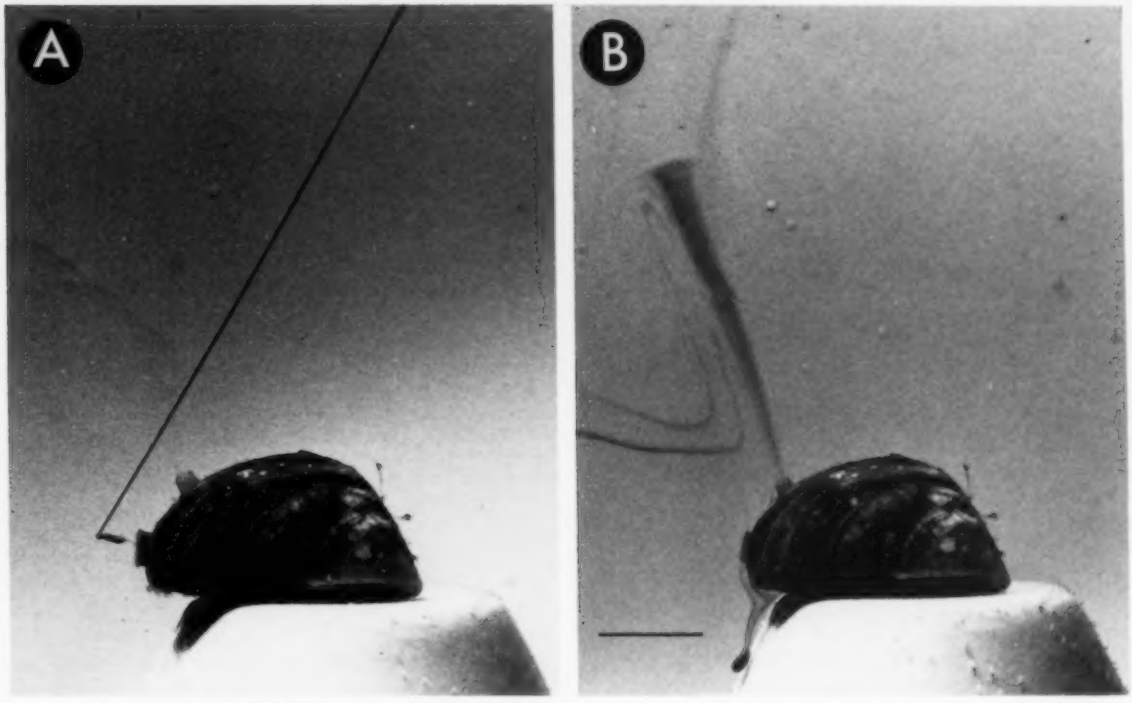

FIG. 2. (A) Dye inhalation into the mantle cavity of a zebra mussel and (B) the subsequent linear and laminar exhalation of the dye. Note the distinct leading edge of the exhalant plume. Bar = 5 mm.

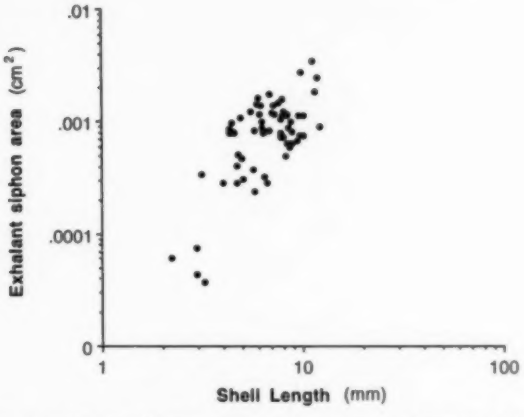

FIG. 3. Exhalant siphon aperture surface area as a function of shell length (Pearson's correlation, $r = 0.57$, $p < 0.0001$, $n = 65$).

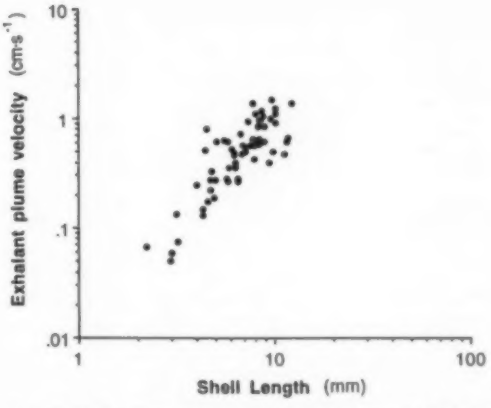

FIG. 4. Exhalant plume velocity determined from video footage of mussels (2–11 mm) pumping dye (Pearson's correlation, $r = 0.71$, $p < 0.0001$, $n = 65$).

both exhalant siphon surface area (Pearson's correlation, $r = 0.57$, $p < 0.0001$, $n = 65$; Fig. 3) and exhalant plume velocity (Pearson's correlation, $r = 0.71$, $p < 0.0001$, $n = 65$; Fig. 4) were positively correlated with mussel shell length. These findings were not surprising, as MacIsaac et al. (1991) reported that exterior inhalant siphon diameter and shell length were positively correlated in *D. polymorpha*. Moreover, the amount of water processed by siphonate bivalves (e.g., *Corbicula, Dreissena*) can increase with mussel shell length (Morton 1971; Leff et al. 1990; Reeders and Bij de Vaate 1990) (Fig. 5) only if exhalant siphon cross-sectional area and/or plume velocity increase concomitantly. Jørgensen et al. (1988) observed that the pumping rate of *Mytilus edulis* was positively correlated with siphon area and was maximal when valves were fully gaped and siphons fully extended.

Pumping rate, determined as the product of exhalant siphon surface area and exhalant plume velocity, was also related to *D. polymorpha* shell length (Fig. 5). The mechanistic basis for

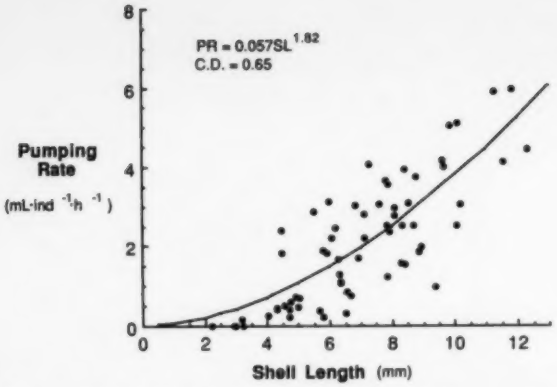

FIG. 5. Relationship between pumping rate and shell length of *D. polymorpha* (2–11 mm shell length) as determined by the dye pumping process. The nonlinear least squares regression line is shown (ANOVA, regression coefficient = 1.82, $p < 0.0005$, $n = 65$). PR = pumping rate, SL = shell length, and C.D. = coefficient of determination.

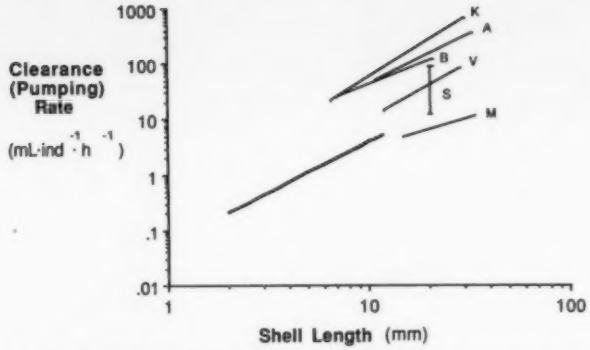

FIG. 6. Regression relationships between adult *D. polymorpha* clearance rate (solid lines) (see Kryger and Riisgård 1988), juvenile *D. polymorpha* pumping rate (broken line; Fig. 5), and shell length. Modified from Kryger and Riisgård (1988). K = original Kryger and Riisgård (1988) data, A = Alimov (1969), B = Kondratev (1963), V = Micheev (1966), S = Stańczykowska (1976) with SE bars, and M = Morton (1971).

this nonlinear relationship is not clear, although Bayne et al. (1976) suggested that it may result from an underlying association between gill area or gill ostia area and animal dry weight.

Our study is the first to assess pumping rate capabilities of Lake Erie *D. polymorpha* < 7 mm. Animals between 2 and 7 mm may process up to 0.2 and 2.0 mL·ind·h^{-1}, respectively (Fig. 5). These values are at least an order of magnitude lower than those of large (>20 mm) *D. polymorpha* (Kryger and Riisgård 1988) (see Fig. 6) but are equal to or greater than individual clearance (=filtering) rates of *Daphnia galeata mendotae* and *D. retrocurva* (0.03–0.47 mL·ind^{-1}·h^{-1} for animals 0.49–1.54 mm in body length), the primary suspension-feeding zooplankters in western Lake Erie (Wu and Culver 1991).

If *D. polymorpha* are 100% efficient at clearing particles >4 μm from suspension, as suggested by Jørgensen et al. (1984), then clearance rate should equal pumping rate. We superimposed our pumping rates on Kryger and Riisgård's (1988) compilation of clearance rate data; similarities and differences exist between the various studies (Fig. 6). For example, our measured and extrapolated pumping rates for individuals between 10 and 12.5 mm shell length are between those of large mussels as measured by Micheev (1966) and Morton (1971) but are considerably lower than those measured by Kondratev (1963), Alimov (1969), and Kryger and Riisgård (1988) for *D. polymorpha* of comparable size (Fig. 6).

Variation in reported clearance rates (Fig. 6) is extensive and possibly the result of a number of factors including temperature (Hinz and Scheil 1972; Jørgensen et al. 1988; Reeders and Bij de Vaate 1990), food type and concentration (Ten Winkel and Davids 1982; Sprung and Rose 1988; Reeders et al. 1989, Reeders and Bij de Vaate 1990), and perhaps experimental method (see review, Kryger and Riisgård 1988). For example, Kryger and Riisgård (1988) speculated that studies conducted with suspensions of graphite particles may have underestimated filtration rate by virtue of the high particle densities used, inefficient retention of particles, or particle unpalatability.

Disturbance associated with handling of mussels prior to experimentation can also affect resultant filtration rates. Reeders et al. (1989) reported that clearance rates continued to increase for at least 24 h after disturbance. This finding contradicts our observation of a rapid response by disturbed mussels. In most instances, experimental animals appeared to fully open valves and extend siphons within minutes (approximately <2–5 min) of disturbance. Coughlan and Ansell (1964) stated that fully extended siphons provide a good indication of pumping characteristics of undisturbed animals. Disturbance (e.g., intensity of culture aeration) during the acclimation period prior to experimentation could influence animal sensitivity during subsequent handling and testing, but its effect has not been addressed. Furthermore, it remains to be determined whether each of the above-mentioned factors, explored with adult *D. polymorpha*, applies to juvenile individuals as well.

Potential Juvenile Mussel Filtering Impact

By combining mean size–frequency distribution data from three reefs in Lake Erie's western basin with the allometric equation for pumping rates (PR = 0.057·SL$^{1.82}$), it is possible to estimate the potential effect that the previously unstudied juvenile mussels have on phytoplankton abundance and, indirectly, on water clarity. Based on observed 1990 densities, *D. polymorpha* with shell lengths between 2 and 11 mm were capable of processing a water column equivalent to the mean depth (approximately 7 m) of western Lake Erie between 0.39 and 0.96 times daily (Table 1). The filtering impact of juvenile and adult *D. polymorpha* should be maximized in western Lake Erie because of the basin's unique morphometry. For example, benthic suspension-feeders probably have access to phytoplankton stocks throughout most of the water column at most times during spring, summer, and fall because the basin rarely stratifies, and stratification only persists for brief intervals when it does occur (Schertzer et al. 1987). The short residence time and shallow mean depth would further serve to minimize the localization of *D. polymorpha* induced phytoplankton depletion to benthic waters. The recent decline in chlorophyll *a* concentration and increase in water clarity (Leach 1992) are consistent with our predictions. While adult mussels probably play a greater role in these changes (MacIsaac et al. 1992), the potential filtering rate of juvenile mussels is considerable and

TABLE 1. Mean density of juvenile *D. polymorpha* (2–11 mm) on three reefs in the western basin of Lake Erie (October 22, 1990) and the potential number of times the entire water column ($\bar{z}$ = 7 m) can be pumped daily (i.e., turnover rate) according to $PR = 0.057 \cdot SL^{1.82}$, where PR = pumping rate ($mL \cdot ind^{-1} \cdot h^{-1}$) and SL = shell length (mm). Mean *D. polymorpha* densities in each 1-mm size class were multiplied by size-specific pumping rates and then summed to yield total potential impact.

Reef	Sites sampled (n)	Mean *D. polymorpha* density ($ind \cdot m^{-2}$)	Water column turnover rate (d^{-1})
Sunken Chicken Is.	8	310 700	0.96
Chickenolee Is.	8	102 550	0.39
Hen Is.	9	226 375	0.82

should not be ignored. These findings are in contrast with those of Wu and Culver (1991) who concluded that *Daphnia* has a greater effect on water clarity and edible algal concentration than *Dreissena*. Our maximum population filtering rate of $0.96 \cdot d^{-1}$ for juvenile *D. polymorpha* inhabiting Sunken Chicken Island Reef (Table 1) is 1.1–4.0 times the maximum 1989 offshore ($0.855 \cdot d^{-1}$) and nearshore ($0.24 \cdot d^{-1}$) rates, respectively, of *Daphnia* (fig 4a and 4b in Wu and Culver 1991). The effects of *Daphnia* grazing are also likely to be far more transient than those of juvenile *D. polymorpha* because of the ephemeral nature of *Daphnia* populations in Lake Erie (Wu and Culver 1991). On the other hand, *Daphnia* residing in the plankton clearly have the potential to influence surface waters whereas the impact of settled *Dreissena* depends on the extent of water column mixing and whether all animals in a colony have full access to the water column.

Other authors have also documented strong filtering impacts of benthic *D. polymorpha* populations. For example, Stańczykowska et al. (1976) reported that littoral populations of *D. polymorpha* at densities of 300 $ind \cdot m^{-2}$ were able to filter the epilimnion of Mikolajskie Lake in Poland twice annually. Moreover, *D. polymorpha* populations in Lakes Stregiel and Taltowisko, Poland, were capable of filtering epilimnetic waters in approximately 5 and 22 d, respectively (Stańczykowska 1968). Hebert et al. (1991) estimated that *D. polymorpha* could filter the entire water column of Lake St. Clair twice daily if densities averaged 6000 $animals \cdot m^{-2}$. Reeders et al. (1989) reported that zebra mussel populations orders of magnitude smaller than those in western Lake Erie were able to filter the water columns of two lakes in The Netherlands in 11 and 18 d. Indeed, rather than being perceived as nuisance organisms, zebra mussels are employed in The Netherlands in programs designed to restore water quality in eutrophic lakes (Reeders and Bij de Vaate 1990). Perhaps the closest analog to *D. polymorpha* filtering capacities in Lake Erie comes from *Mytilus* populations in a Danish fjord; Jørgensen (1980) suggested that these mollusc aggregations were capable of filtering the water column 10 times per day. However, because thermal and salinity stratification precluded thorough water column mixing, these mussels were probably limited to refiltering benthic waters many times per day.

We conclude that juvenile *D. polymorpha* have a great potential to filter phytoplankton from, and increase the transparency of, the water column of western Lake Erie. This potential results from the enormous mussel densities in western Lake Erie and from the modest per capita pumping rates of individuals between 2 and 11 mm.

Acknowledgements

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Sediment Resuspension, Redeposition, and Focusing in a Small Dimictic Reservoir

William F. James

Waterways Experiment Station, Eau Galle Limnological Laboratory, P.O. Box 237, Spring Valley, WI 54767, USA

and John W. Barko

Waterways Experiment Station, Environmental Laboratory, P.O. Box 6199, Vicksburg, MS 39180-6199, USA

James, W.F., and J.W. Barko. 1993. Sediment resuspension, redeposition, and focusing in a small dimictic reservoir. Can. J. Fish. Aquat. Sci. 50: 1023–1028.

Rates of seston (dry mass) sedimentation, estimated from sediment traps (ST rates) and sediment cores (SC rates), were determined at four water depths to examine seasonal and annual sedimentation patterns in Eau Galle Reservoir, Wisconsin (USA). Annual ST rates overestimated annual SC rates at water depths ≤4 m, suggesting that sediment resuspension and redeposition in relatively shallow regions of the reservoir caused the overestimate. In contrast, annual ST and SC rates were similar at depths >4 m, suggesting minimal sediment resuspension and redeposition in deeper regions of the reservoir. Using variations in daily ST rates estimated during the autumn overturn of 1987, we developed a conceptual framework for estimating sediment resuspension, redeposition, and focusing. Resuspension of shallow sediments during this particular period accounted for over 50% of the annual ST rate at depths ≤4 m. At the same time, focusing of resuspended shallow sediments to deeper regions accounted for about 20% of the annual ST rate at depths >4 m. Our results indicate that during autumn overturn in this reservoir, peaks in sedimentation in the deep, profundal region, estimated from sediment traps, may reflect sediment focused from shallow regions rather than sediment resuspended from the profundal region.

Les vitesses de sédimentation du seston (masse sèche), estimées à partir de pièges de sédimentation (vitesses PS) et de carottes de sédimentations (vitesses CS), ont été mesurées à quatre profondeurs différentes pour étudier les caractéristiques de la sédimentation saisonnière et annuelle dans le réservoir Eau Galle, Wisconsin (É.-U.). Les vitesses PS annuelles ont entraîné une surestimation des vitesses CS à des profondeurs ≤ 4 m, ce qui laisserait supposer que la remise en suspension et la redéposition des sédiments dans les régions relativement peu profondes du réservoir seraient à l'origine de la surestimation. À l'opposé, les vitesses PS et CS annuelles étaient semblables à des profondeurs > 4 m, indiquant une remise en suspension et une redéposition minimales des sédiments dans les régions plus profondes du réservoir. À l'aide des variations des vitesses PS quotidiennes estimées au cours du brassage convectif d'automne de 1987, nous avons mis au point un cadre conceptuel pour estimer la remise en suspension, et la redéposition et la concentration des sédiments. La remise en suspension des sédiments dans les eaux peu profondes durant cette période particulière compte pour plus de 50 % de la vitesse PS annuelle à des profondeurs ≤ 4 m. En même temps, la concentration des sédiments remis en suspension dans les eaux plus profondes compte pour environ 20 % de la vitesse PS annuelle à des profondeurs > 4 m. Nos résultats indiquent qu'au cours du brassage convectif d'automne dans ce réservoir, les maximums de sédimentation dans le région profonde, estimés à partir des pièges à sédimentation, peuvent refléter les sédiments concentrés depuis les régions peu profondes, plutôt que les sédiments remis en suspension depuis la région profonde.

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The occurrence of greater rates of sediment accumulation in deeper than in shallow regions of lakes has been attributed to erosional mechanisms that collectively result in sediment resuspension and focusing (Davis 1968, 1973; Davis and Brubaker 1973; Likens and Davis 1975; Håkanson 1977). Many studies conducted in the profundal region of lakes using sediment traps have documented exceptionally high peaks in sedimentation rates during spring and/or autumn overturn (Pennington 1974; White and Wetzel 1975; Lastein 1976; Davis et al. 1984) as a result of sediment resuspension and focusing. However, these processes have been very difficult to quantify (cf. Davis et al. 1984; Hilton 1985; Hilton et al. 1986), due to difficulties in determining with certainty the origins of settling seston (i.e., from the littoral, profundal, or a combination) collected by sediment traps located in the profundal region. Studies that quantify sediment resuspension and focusing are needed in the evaluation of nutrient budgets (Dillon et al. 1990; Caraco et al. 1992; James and Barko 1992) and spatial and depth-related variations in sedimentation (Hilton et al. 1986).

Davis (1973) and Davis and Brubaker (1973) suggested that sediment is resuspended primarily from shallow sediments, mixed throughout the water column, and deposited uniformly over the lake basin during overturn periods, resulting in focusing of shallow sediments to deeper regions. Evaluations of wave dynamics and sediment scour in relation to basin morphometry appear to support these contentions (Carper and Bachmann 1984; Bengtsson et al. 1990) and may explain the depth-related changes in both sediment accumulation and composition in lakes (Håkanson 1977; Kamp-Nielson and Hargrave 1978; Evans and Rigler 1980, 1983; Murchie 1985; Hilton et al. 1986;

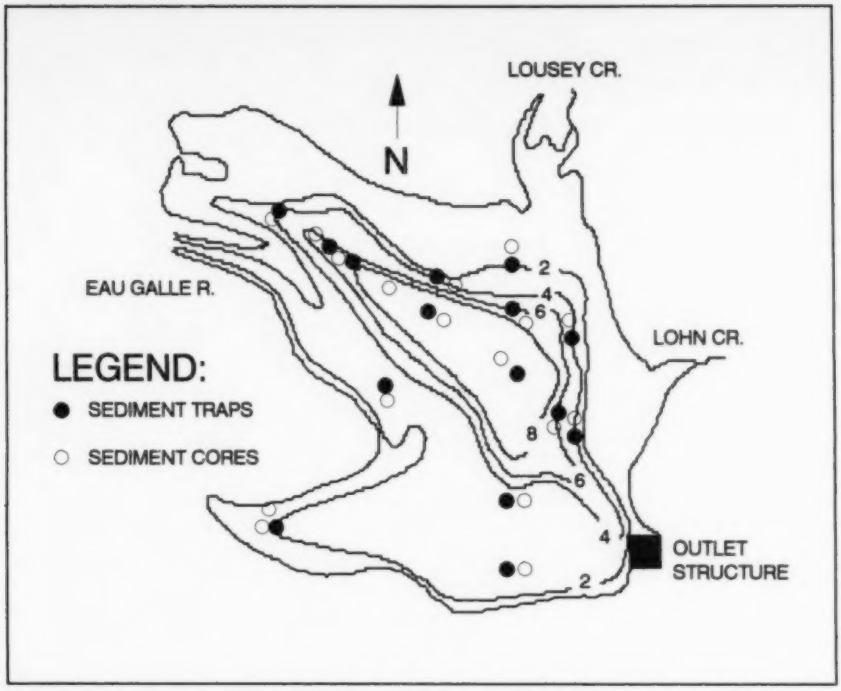

FIG. 1. Bathymetric (m) contours and sediment trap and sediment core stations in Eau Galle Reservoir.

James and Barko 1990). Whereas autumnal peaks in sedimentation rates estimated from sediment traps in the profundal region may result from sediment focusing, more evidence is needed to quantify this sediment transport process.

Hilton (1985) suggested that if sediment resuspension and focusing are occurring during overturn periods, sediment traps should measure greater rates of sedimentation than sediment cores because sediment traps can measure sediment resuspension, while sediment cores cannot measure this process. While this hypothesis provides a good basis for quantifying sediment resuspension and possibly focusing, few studies have directly compared sedimentation rates estimated from sediment traps and sediment cores. Here, we compare sedimentation rates estimated from both sediment traps and sediment cores at several depths in Eau Galle Reservoir, Wisconsin (USA), to discern regions of sediment resuspension and focusing and to suggest possible origins of redistributed sediment (i.e., littoral and/or profundal depths). Based on these comparisons, we estimate rates of sediment transport during a period of autumn overturn in 1987.

Methods

Study Site

Eau Galle Reservoir (Fig. 1), located in west-central Wisconsin (USA), is dimictic, small (0.6 km^2), and has mean and maximum depths of 3.2 and 9 m, respectively. The basin is sinusoidal in shape, with over 60% of the reservoir bottom occurring at depths of 4 m or less. The bottom slopes most steeply at the dam face and around the central region of the reservoir between the 4- and 6-m depth contours (Fig. 1), although the slope is <14%. A zone of sediment erosion, occupying 26% of the reservoir surface area, is located at depths ≤4 m in nonvegetated regions of the reservoir (James and Barko 1990). Surficial sediments in this zone exhibit a mean moisture content of 47% and a sediment density of 0.78 $g \cdot mL^{-1}$, which contrasts markedly with a mean moisture content and sediment density of 76% and 0.23 $g \cdot mL^{-1}$, respectively, for those sediments located at depths greater than about 7 m (James and Barko 1990).

Definition of Sedimentation Variables

Resuspended sediment represents sediment that is mixed into the water column during wind-generated mixing events. Resuspension can occur as a result of wave action (Håkanson 1977; Carper and Bachmann 1984; Bengtsson et al. 1990) and water column mixing during periods of overturn. *Focused sediment* is defined as resuspended sediment that is transported from shallow to deeper regions of lakes during sediment resuspension events, as described by Likens and Davis (1975) and Håkanson (1977). Sediment focusing is likely to occur when resuspended sediment originating from shallow regions is homogeneously mixed throughout the lake basin during wind-generated mixing events. Slumping or sliding of sediments from steeply sloping basins can also result in sediment focusing (Håkanson 1977). *Redeposited sediment* is defined as that resuspended sediment that settles during quiescent periods back to region where it originated (Bloesch 1982; Bloesch and Uehlinger 1986).

We define *net sedimentation* as sediment originating from outside the lake basin (i.e., from the watershed or autochthonous production) that accumulates on a lake bottom and remains in the same region over a defined time period such as a year.

TABLE 1. Comparison of grand mean (±1 SE) annual ST and SC rates ($kg \cdot m^{-2} \cdot yr^{-1}$) at four depths in Eau Galle Reservoir. Annual ST rates were calculated for the periods of April 1986–87 and November 1986–87. Asterisks represent significant differences between ST and SC rates ($p < 0.05$; t-test). Different letters indicate significant differences at the 5% level or less (Duncan's multiple range analysis) between depths. Reservoir-wide ST and SC rates were calculated as the sum of rates integrated over areas (m^2) enclosed by each depth range.

Method	Water depth (m)				Reservoir-wide rate
	2	4	6	8	
ST rate	3.5 (0.3)*	4.2 (0.2)*	6.1 (0.3)	9.6 (2.4)	5.5
n	4	12	8	4	
ANOVA	c	bc	b	a	
SC rate	2.3 (0.2)	1.9 (0.6)	6.9 (0.8)	10.5 (0.9)	4.6
n	3	6	4	3	
ANOVA	c	c	b	a	
Area	144 405	229 783	107 631	121 184	603 003

Gross sedimentation represents net sedimentation plus sediment redistributed via sediment resuspension/redeposition/focusing processes.

Sediment Trap and Sediment Core Collection Methods

Sedimentation rates estimated from sediment traps (ST rates) were measured at biweekly to monthly intervals from May 1986 to November 1987. The sediment traps were deployed 0.5 m above the bottom at depths of 2 m (two stations), 4 m (seven stations), 6 m (four stations), and 8 m (two stations) at various stations located around the reservoir (Fig. 1). The sediment traps consisted of three replicate cylinders, each having an aspect ratio of 5.0 (50 cm length/10 cm diameter; Lau 1979; Bloesch and Burns 1980), attached to a carousel frame. Carousels were held vertically in the water column by a buoy suspension system. Samples from each cylinder were thoroughly homogenized and subsampled for the determination of dry mass. Dry mass was determined on glass fiber filters dried to a constant weight at 105°C. Daily ST rates were calculated as dry mass divided by the product of the cross-sectional area of the cylinder and days of deployment. Annual ST rates were calculated separately for the periods April 1986–87 and November 1986–87 as the sum of daily ST rates over each deployment period.

During the winters of 1986 and 1987, sediment cores were collected under ice near the sites of sediment trap deployment (Fig. 1). Sediment cores were collected with a gravity core sampler (Wildco Wildlife Supply) equipped with plastic core liners (5.1 cm inside diameter and 91.4 m length). Each core sample was sectioned at 5-cm intervals until preimpoundment soils were encountered (James and Barko 1990). Core sections above the preimpoundment soil were dried at 105°C for dry mass determination. Annual rates of sedimentation estimated from sediment core records (SC rates), representing an average sedimentation rate over 17 yr (i.e., reservoir age), were calculated as the sum of dry mass above preimpoundment soils divided by the product of cross-sectional area of the core liner and reservoir age.

Air temperature and wind speed were monitored each hour with a recording thermistor probe and anemometer (OmniData International) during a period of cooling and autumnal overturn in 1987 (August–November) to evaluate meteorological influences on daily ST rates estimated during the same period. Measurements of water temperature were taken at 0.50-m intervals from the reservoir surface to the bottom during the same period.

TABLE 2. Comparison of daily summer (1 June – 30 September) versus daily autumnal (1 October – 30 November) ST rates ($g \cdot m^{-2} \cdot d^{-1}$), integrated over time, at four depths in 1987. Integration over time provided average daily ST rate estimates.

Period	Water depth (m)			
	2	4	6	8
Summer	11.2	10.3	15.6	19.8
Autumn	27.8	27.7	38.9	51.0

Results

Mean annual ST and SC rates varied as a function of water depth (Table 1), with rates increasing with increasing depth, particularly at depths >4 m. Annual ST rates were significantly greater ($p < 0.05$; t-test) than annual SC rates at depths ≤4 m. In contrast, annual ST and SC rates were similar at depths >4 m. Reservoir-wide ST rates, integrated over sediment area, overestimated reservoir-wide SC rates by 0.9 $kg \cdot m^{-2} \cdot yr^{-1}$, due primarily to discrepancies at depths ≤4 m (Table 1).

Since sediment resuspension, redeposition, and focusing (potentially accounting for discrepancies between ST and SC rates) are likely to occur during periods of overturn, we examined weather and water temperature patterns in relation to daily ST rates during the autumn overturn period of 1987. Prior to overturn in 1987, water temperatures cooled and stratification weakened during August–September, coinciding with steady decreases in mean daily air temperature; isothermal conditions existed by mid-October (Fig. 2). Increases in mean daily wind speed and decreases in mean daily air temperature occurred during October. Water column temperatures continued to cool and remained isothermal during October–November.

Mean daily ST rates were relatively low during the summer stratified period (i.e., June–September) and increased to a peak during autumn overturn (Fig. 3). In particular, greatest mean daily ST rates, occurring in late September, coincided with the period when maximum wind speeds exceeded 30 $km \cdot h^{-1}$. During the summer stratified period, average daily ST rates were similar at 2 and 4 m and exhibited slight increases with increasing depth at depths >4 m (Table 2). In contrast, daily ST rates

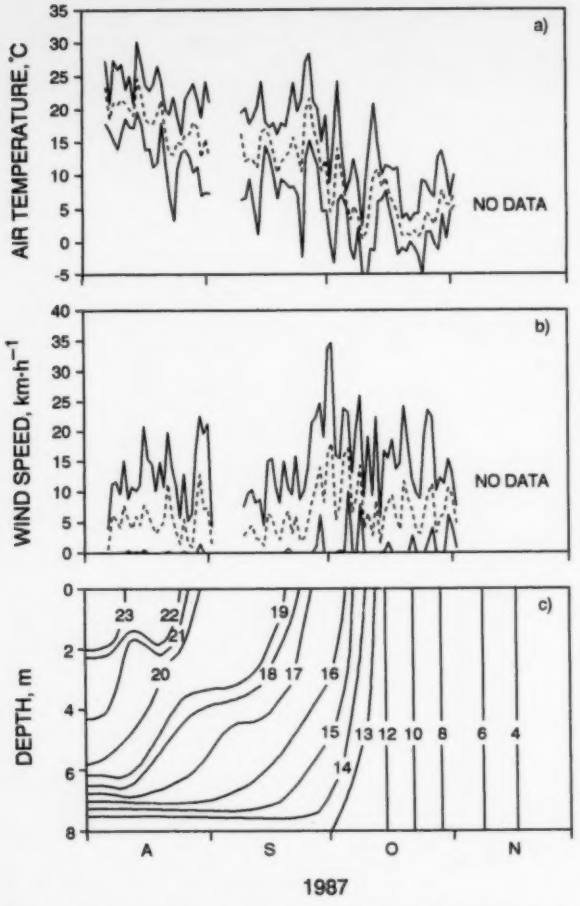

FIG. 2. Seasonal variations in (a) mean, minimum, and maximum daily air temperature, (b) mean, minimum, and maximum wind speed, and (c) water temperature (°C) contour lines in Eau Galle Reservoir during late summer and autumn 1987.

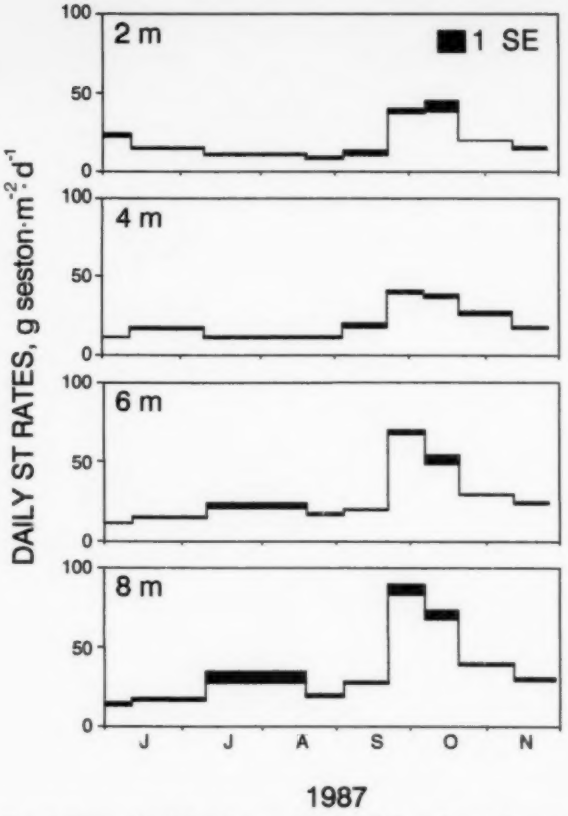

FIG. 3. Seasonal variations in mean (± 1 SE) daily sedimentation rates estimated from sediment traps (ST rates) at four water depths during summer and autumn 1987.

at all depths were much greater during autumn overturn than during the summer stratified period in 1987, particularly at depths >4 m (Table 2). However, the percent increase from summer to autumn was similar at each depth (~160% increase). This pattern suggested that suspended sediment was distributed uniformly throughout much of the vertical water column during both time periods, although concentrations of suspended sediment were much greater during autumn overturn, as a result of sediment transport processes.

A conceptual framework, similar to the one suggested by Davis and Brubaker (1973), was developed to characterize spatial patterns of sediment resuspension, redeposition, and focusing during autumn overturn. In our conceptual framework, we assumed that sediment resuspension occurred primarily at depths ≤4 m and that a portion of this resuspended sediment was redeposited over the same depth range while another portion was focused to depths >4 m. Increases in daily ST rates at depths ≤4 m during autumn overturn (Table 2) were apparently the result of sediment redeposition, since annual ST rates significantly overestimated annual SC rates at these depths (Table 1). Increases in daily ST rates at depths >4 m during autumnal overturn were assumed to result from sediment focusing only and not from sediment resuspension and redeposition of profundal sediments, since annual ST rates were very similar to annual SC rates at these depths (Table 1). Had sediment resuspension and redeposition occurred during autumn overturn at depths >4 m, we would have expected annual ST rates to be greater than annual SC rates, as observed at depths ≤4 m. For all these sedimentation processes to occur, it was further assumed that homogeneous mixing of resuspended sediment particles occurred throughout the water column.

We estimated sediment focusing during autumn overturn of 1987 as the difference between average daily summer and autumn ST rates at depths >4 m (Table 2) and assumed that the daily summer ST rates represented net sedimentation during autumn overturn as well (i.e., baseline method of Håkanson et al. 1989). The rate differences, representing sediment focusing alone, were then integrated over reservoir areas below 4 m, multiplied by the estimated duration of autumn overturn (i.e., 61 d, 1 October to 30 November), and normalized with respect to reservoir surface area (0.6 km^2) to estimate the mass of sediment focused during the entire autumn overturn period. This rate could then be directly compared with the annual SC rates estimated at the same depth. The estimated sediment focusing rate of 0.6 $kg \cdot m^{-2}$ during autumn overturn accounted for about 20% of the annual SC and ST rate at depths >4 m (Fig. 4).

A sediment redeposition rate during autumn overturn of

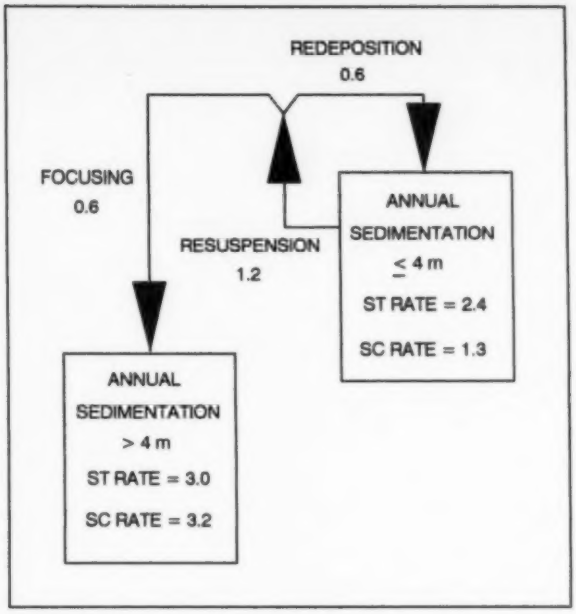

FIG. 4. Sediment resuspension, redeposition, and focusing in shallow and deep regions of Eau Galle Reservoir during autumn overturn, 1987. Annual sedimentation rates estimated from sediment traps (ST rates) and sediment cores (SC rates) are provided for comparison. All data ($kg \cdot m^{-2} \cdot yr^{-1}$) are normalized with respect to the reservoir surface area (0.6 km^2).

0.6 $kg \cdot m^{-2}$ (Fig. 4) was also estimated using differences between daily summer and autumn ST rates for depths ≤4 m (Table 2), normalized with respect to reservoir surface area. The similarity between the sediment focusing and sediment redeposition rate resulted from normalization of the two rates with respect to reservoir surface area. Sediment resuspension during autumn overturn, calculated as the sum of sediment focusing and redeposition rates, was 1.2 $kg \cdot m^{-2}$ (Fig. 4). In essence, sediment resuspension accounted for (1) sediment collected by sediment traps during the summer stratified period that was later focused during autumn overturn and (2) sediment redeposited into sediment traps during autumn overturn. The sediment resuspension rate accounted for 50% of the annual ST rate at depths ≤4 m (Fig. 4).

We corrected reservoir-wide annual ST rates (Table 1) using the sediment resuspension rate during autumn overturn (Fig. 4), which allowed direct comparison with reservoir-wide annual SC rates. The corrected reservoir-wide annual ST rate of 4.3 $kg \cdot m^{-2} \cdot yr^{-1}$ was much closer to the reservoir-wide annual SC rate of 4.6 $kg \cdot m^{-2} \cdot yr^{-1}$, indicating that sediment resuspension at depths ≤4 m during autumn overturn esentially accounted for all of the discrepancy between annual ST and SC rates.

Discussion

Analyses of daily ST rates, and annual ST and SC rates, allowed for the discrimination of distinct regions of sediment resuspension, redeposition, and focusing in Eau Galle Reservoir. Processes of sediment resuspension and redeposition were confined to relatively shallow depths whereas sediment focusing occurred in the deep basin of this reservoir. Thus, autumn peaks in daily ST rates, relative to the summer, were the result of sediment redeposition at shallow depths and sediment focusing at deeper depths. These results lend support to the conceptual model of sediment resuspension and focusing developed by Davis (1973) and Davis and Brubaker (1973), suggesting that sediment is resuspended primarily from shallow areas and focused to deeper areas during autumn overturn in small lakes. They also support the occurrence of an erosional zone in Eau Galle Reservoir at nonvegetated depths ≤4 m (James and Barko 1990).

Our results provide strong evidence that sediment cores and sediment traps measure different components of annual sedimentation along a depth gradient in Eau Galle Reservoir. At shallow depths, data from sediment cores reflect net annual sedimentation, while data from sediment traps reflect gross annual sedimentation. Because sediment traps are used primarily to measure short-term sedimentation rates (i.e., they are retrieved at weekly to biweekly intervals), they measure the sedimentation of particles that can be resuspended from the reservoir bottom and redeposited at a later date. Thus, sediment traps appear to inherently overestimate net sedimentation rates at shallow depths in this reservoir. At deeper depths, data from both sediment cores and sediment traps reflect gross sedimentation, due to sediment focusing processes.

Whereas annual ST rates are representative of only one point in time, SC rates in contrast represent a mean measure of sedimentation that may be quite variable annually over a period of several decades. Thus, caution is suggested when interpreting discrepancies between annual ST and SC rates. In our study, mean annual discharge from the reservoir ($3.09 \times 10^7 \cdot m^{-3} \cdot yr^{-1}$) during the sediment trap deployment period of 1986–87 was nearly the same as the mean annual discharge calculated over the 18-yr period of reservoir existence ($3.05 \times 10^7 \cdot m^{-3} \cdot yr^{-1}$) spanned by the sediment cores. Therefore, average sediment loading was probably quite similar between the two time frames, allowing for direct comparisons between annual ST and SC rates.

Redeposition during autumn overturn represented 46% of the annual net sedimentation rate (i.e., annual SC rate) at shallow depths (i.e., Fig. 4). Although we assumed that sediment traps accurately estimated sediment redeposition in shallow regions, there is no evidence in the literature to support this assumption. Indeed, many reviews and evaluations have strongly suggested that sediment traps perform poorly in turbulent waters (Bloesch and Burns 1980; Butman 1986; Butman et al. 1986). Having estimated sediment redeposition from sediment trap data, we acknowledge that this measure must be corroborated with independent measurements from sediment budgets based on detailed observations of changes in suspended sediment concentration in the water column both during and after water column mixing periods (i.e., Kristensen et al. 1992).

Hilton et al. (1986) suggested that sediment resuspension occurred over the entire lake basin of Esthwaite Water during overturn periods, which contrasts with our observation that only shallow sediments are being resuspended in Eau Galle Reservoir. We suggest, as did Hilton (1985), that depths where sediment resuspension occur may vary between lakes as a function of basin morphometry, exposure to winds, and effective fetch. In lakes with small effective fetches, such as Eau Galle Reservoir, sediment resuspension may be confined primarily to shallow depths where wave scour and other water turbulence is greatest. As the effective fetch increases, other physical mech-

anisms of water turbulence such as seiche activity, metalimnetic upwelling during high wind stress, and billowing during metalimnetic erosion (see Imberger and Patterson 1990) may become increasingly more important at deeper depths, causing resuspension of sediments throughout the entire lake basin. Thus, more information is needed to discern differences in depths of sediment resuspension among different lake types. Investigations of sediment transport processes using sediment traps, sediment cores, and independent tracers need to be conducted under a variety of morphometric and climatic conditions, and compared with physical mechanisms causing water turbulence, to more clearly understand sedimentation and sediment redistribution phenomena.

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On the Statistical Distribution of Salmon in the Sea: Application of the Negative Binomial Distribution, and the Influence of Sampling Effort

David W. Welch

Department of Fisheries and Oceans, Pacific Biological Station, Nanaimo, B.C., Canada V9R 5K6

and Yukimasa Ishida

National Research Institute of Far Seas Fisheries, 7-1, Orido 5-chome, Shimizu-Shi, Shizuoka, Japan 424

Welch, D.W., and Y. Ishida. 1993. On the statistical distribution of salmon in the sea: application of the negative binomial distribution, and the influence of sampling effort. Can. J. Fish. Aquat. Sci. 50: 1029–1038.

We examined the sampling statistics of Pacific salmon (*Oncorhynchus* spp.) caught in a series of experimental gillnet sets on the high seas and demonstrate how the reliability of the catch statistics varies with the amount of sampling effort. Our analysis indicates that the replicate catches, which were made under essentially identical conditions, are adequately described by the negative binomial distribution. We also extend the utility of this distribution for describing catch statistics by showing that (1) the shape parameter of the distribution can be directly interpreted as the number of degrees of freedom (df) associated with each observation of catch and (2) the df are related to the amount of sampling effort used and the size of the biological aggregation being encountered by the gear. For salmon caught on the high seas, approximately 1 df is obtained per 15 m of gill net used. Identification of the statistical model describing the uncertainty in high-seas gillnet catches should help in the design of better high seas surveys and in the analysis of historical data bases. As an example, we show how the statistical certainty associated with estimates of salmon density changes with the amount of sampling effort applied.

Nous avons examiné les données statistiques sur l'échantillonnage du saumon du Pacifique (*Oncorhynchus* spp.) capturé au cours d'une série de pêches expérimentales au filet maillant en haute mer et démontré que la fiabilité des données statistiques sur les prises varie avec l'intensité de l'effort d'échantillonnage. Notre analyse indique que les prises répétées, qui ont été réalisées essentiellement dans les mêmes conditions, sont décrites de façon appropriée par la distribution binomiale négative. Nous avons également étendu l'utilité de cette distribution à la description des données sur les prises en montrant que 1) le paramètre qui donne la forme de la distribution peut être interprété directement comme le nombre de degrés de liberté (dl) associé à chaque observation des prises et 2) les dl sont liés à l'intensité de l'effort d'échantillonnage et à la taille de l'agrégation biologique rencontrée par l'engin de pêche. Pour le saumon capturé en haute mer, on obtient environ 1 dl pour 15 m de filet maillant utilisé. La reconnaissance du modèle statistique qui décrit l'incertitude concernant les prises au filet maillant en haute mer devrait nous aider à concevoir de meilleurs relevés en haute mer et à analyser les bases de données historiques. À titre d'exemple, nous montrons comment la certitude statistique liée à l'estimation de la densité du saumon varie avec l'intensité de l'effort d'échantillonnage appliqué.

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Gill nets or trawls are widely used in the North Pacific Ocean to estimate the abundance and distribution of Pacific salmon. For many years, Japan has conducted multiship research surveys to assess the abundance of Pacific salmon and to study aspects of the basic biology of salmon on the high seas. Many Pacific Rim countries also use similar gill nets for forecasting the strength of returning salmon runs in their coastal waters. However, before these data can be reliably used, the sampling characteristics of both salmon and the fishing gear need to be assessed.

This study examines one aspect of the scientific study of high-seas salmon biology: what the inherent statistical uncertainty is in a single catch observation made on the high seas. To determine this uncertainty, we examined the frequency (or sampling) distribution of a replicate series of catches made under constant conditions. Without adequate information on the nature of this sampling distribution, it is difficult to accurately weigh the reliability inherent in the point estimates of catch so obtained. Establishing this information is also important in coastal waters, where the results from gillnet surveys are used as predictors of salmon abundance and the results from trawl surveys are used to establish the abundance of exploited groundfish stocks.

The study of the variability of fish catches and other aquatic organisms has a long history. Studies on replicated plankton catches showed that the Poisson model was an inadequate model because the variability between samples increased faster than the mean when replicate samples taken at different plankton densities were compared (the variance is directly proportional to the mean for the Poisson distribution). This "excess" variability was attributed to variation in the volume of water actually sampled.

Taylor (1953) examined the variability of replicate trawl samples for groundfish on Georges Bank. He also found that the

catch data were not satisfactorily described by a Poisson distribution because, as with plankton samples, there was a greater preponderance of very small or very large samples than predicted by the Poisson. He concluded that the negative binomial distribution might be an adequate model of the uncertainty intrinsically present in any given observation of catch because it had longer tails. However, he also concluded that the variability in excess of that expected from the Poisson distribution could be due to spatial heterogeneity in the sampled population.

Clearly, variation in both the amount of sampling effort (i.e., the volume of water filtered through a plankton net, or the area swept by a trawl) as well as spatial heterogeneity of the organism can contribute to extra-Poisson variability. However, this raises an important question: if one sample is based on a catch obtained using twice as much sampling effort as another, it is presumably more statistically reliable. But more reliable than what, and what minimal amount of sampling effort forms a fundamental unit of statistical observation? Current use of fishing surveys routinely normalize the catches to form a measure of catch-per-unit-effort that takes into account variation in fishing effort, but does not provide any insight into the effect of fishing effort on statistical reliability.

A coherent view of the relative effects of sampling effort and spatial heterogeneity on the reliability of a set of catch observations is lacking in the fisheries literature. Yet this understanding is crucial for attempts to assess the uncertainty inherent in fisheries surveys and for weighting the information that comes from different survey techniques.

We consider two closely related issues in this paper. First, we examine the information present in the results from a single fishing operation. Normally, a single fishing operation can be plausibly viewed as providing one observation, or one degree of rreedom (df), on fish density or abundance. However, if a single operation provides more information than 1 df about fish abundance, ignoring this information is wasteful and incorrect. To examine this question, we establish the length scale at which spatially separated samples can be considered uncorrelated, and therefore independent.

Second, we ask what statistical distribution the data can reasonably be considered to have been drawn from. Knowing the degrees of freedom that a specific fishing gear can provide, plus the underlying distribution that the observations are drawn from, allows a useful parsimony in describing the uncertainty associated with a given set of data. This can contribute to both improved survey designs (e.g., Pennington and Vølstad 1991) and more accurate methods of statistical analysis.

Methods

A series of 30 gillnet experiments were conducted using the Japanese Fisheries Agency's research vessel *Wakatake Maru*, with 19 experiments done at night between 10 May and 6 June 1983 in the western North Pacific and 11 experiments done in the Bering Sea between 19 and 31 July 1984. Fishing was not conducted on all nights within these periods. Each experiment consisted of measuring the number of salmon caught by consecutive 5-tan (approximately 250 m) sections of a 100-tan (5-km) commercial gill net. Three salmon species (sockeye, *Oncorhynchus nerka*; chum, *O. keta*; and pink, *O. gorbuscha*) were caught in sufficient abundance to be separately analyzed. The data therefore consist of 90 separate experiments (30 nights fishing × 3 species), with each experiment consisting of 20 replicate catches made under essentially identical conditions. We excluded one data set where no pink salmon were caught, leaving 89 useful experiments. Further details on the 1984 experiments were described by Ishida (1986).

Set locations are shown in Fig. 1. A Japanese commercial gill net with 121-mm mesh size was used for the experiments. Soak times ranged from 11 h 18 min to 12 h 16 min in 1983, with most nearly 12 h in length. Soak times in 1984 ranged from 10 h 51 min to 11 h 36 min, with most over 11 h. Because we do not make comparisons of catch magnitude between days in this paper, we ignored the effect of differences in soak time on catch levels and worked directly with the raw data. Average catch levels were substantially higher during the 1984 Bering Sea experiments than during the 1983 experiments in the Pacific Ocean.

At the end of each night's fishing the net was retrieved and the number of salmon caught per 5-tan section was recorded for each species. A total of six corrections were subsequently made to the 30 recorded data sets. On one occasion, a 1-tan panel within a 5-tan section broke, and only the catch by the other four panels was recorded. We inflated the catch by 1.25 and rounded the value to correct for the lost gear. On three separate nights the catch of two adjacent 5-tan panels was accidentally recorded together. In this case, we divided the recorded catch by species equally between the two 5-tan groups. (When odd numbers occurred, the last fish was added randomly to one 5-tan section to maintain integer catches.) Finally, on two occasions the catch from a 5-tan section of net was not recorded, and the oversight was not recognized until the net had been completely recovered. It was not possible to identify which of the other sections had a double count because of the large variability between sections. For these two experiments, we based our statistical analysis on 19 observations rather than 20.

Statistical Distribution of Catches

Spatial Autocorrelation

How much information is contained in one fishing operation? Normally, a single tow of a plankton or trawl net, or a fixed period of fishing with a gill net, is considered to provide one observation on abundance or density. A check on this assumption can be made by calculating the correlation of the catches in adjacent 5-tan sections of the net. Calculating the spatial autocorrelation estimates for individual experiments and then averaging over the 30 separate experiments (Fig. 2) demonstrate that catches in net sections separated by 250 m or more are uncorrelated. It is therefore possible to treat the catches in the 20 sections as statistically independent replicates drawn from the same underlying sampling distribution.

Mean versus Variance

The 20 individual catch observations per 5-tan section can therefore initially be treated as each being based on 1 df and the 20 subcatches from a night's fishing used to calculate the mean catch, μ, and its associated variance, σ^2. A plot of the variance versus the mean (Fig. 3) indicates that the variance increases faster than the mean with increasing fish density, as roughly $\sigma^2 \approx \alpha \cdot \mu^{1.2 \pm 0.03}$, and that this relationship is similar for all three species (Table 1). A similar supralinear increase in variance with the mean was shown by Fukuhara (1975) for sockeye salmon caught on the high seas and by Taylor (1953) for replicate trawl catches for a number of demersal groundfish species.

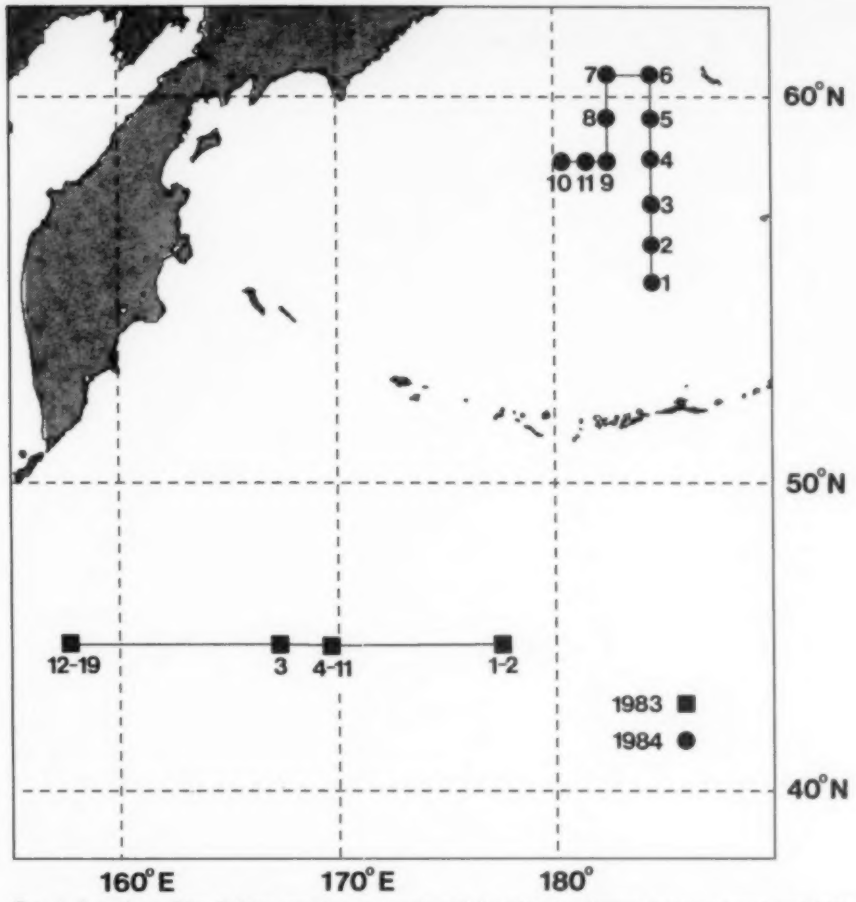

FIG. 1. Location of the fishing experiments conducted in 1983 (northwest Pacific Ocean) and 1984 (Bering Sea), with the sequence of sampling locations indicated.

TABLE 1. Least-squares estimates of the parameters describing the increase in variance with the mean (± 1 SE).

	α	β	r^2
Sockeye	0.09 ± 0.07	1.18 ± 0.04	0.97
Chum	0.31 ± 0.25	1.13 ± 0.09	0.85
Pink	0.14 ± 0.08	1.18 ± 0.07	0.91
Aggregate	0.13 ± 0.05	1.19 ± 0.03	0.96

Table 2 lists the properties of a number of potentially useful statistical distributions where the variance increases as a function of the mean. In each case the distributional definition has been given in terms of the mean, μ, and (for the negative binomial and gamma distributions) a second parameter, k. Note that the variance for a Poisson process is always equal to the mean, while the variance for the exponential and the gamma distributions always increases proportional to the mean-squared. The variance for the negative binomial increases at an intermediate rate, which depends on the value of k; we make use of these facts below. As the degrees of freedom increase, the negative binomial distribution approaches the Poisson, as suggested by the formula for the variance (Table 2).

The parameter k of the negative binomial has varying interpretations between statistical and biological disciplines. In ecology, k has been used as an index of aggregation, with higher values indicating progressively less clumped spatial distributions. In the statistical literature, however, k has been viewed simply as a shape parameter that controls the overall shape of the distribution, with higher values indicating progressively less overdispersion relative to the Poisson.

A simpler and more consistent interpretation can be made, however, by noting that the sum of two or more independent negative binomial random variables $NB(\mu,k_1)$ and $NB(\mu,k_2)$ is still distributed as a negative binomial, but as $NB(\mu,k_1 + k_2)$ (Johnson and Kotz 1969, p. 124). (A similar argument can also be made for the gamma distribution, which can be derived as the sum of k independent exponentially distributed random variables, each with mean μ.) Thus for either distribution drawing an additional (independent) sample from the same population contributes one additional observation, thereby justifying the interpretation of k as the degrees of freedom associated with the sample mean. For example, if $k = 1$ for a particular fishing gear fished for 1 h, then the same gear fished for 2 h should therefore provide 2 df if gear saturation does not occur, and the catch in each 1-h period is independent. Although in each case, only one observation of catch is made, the observation in the

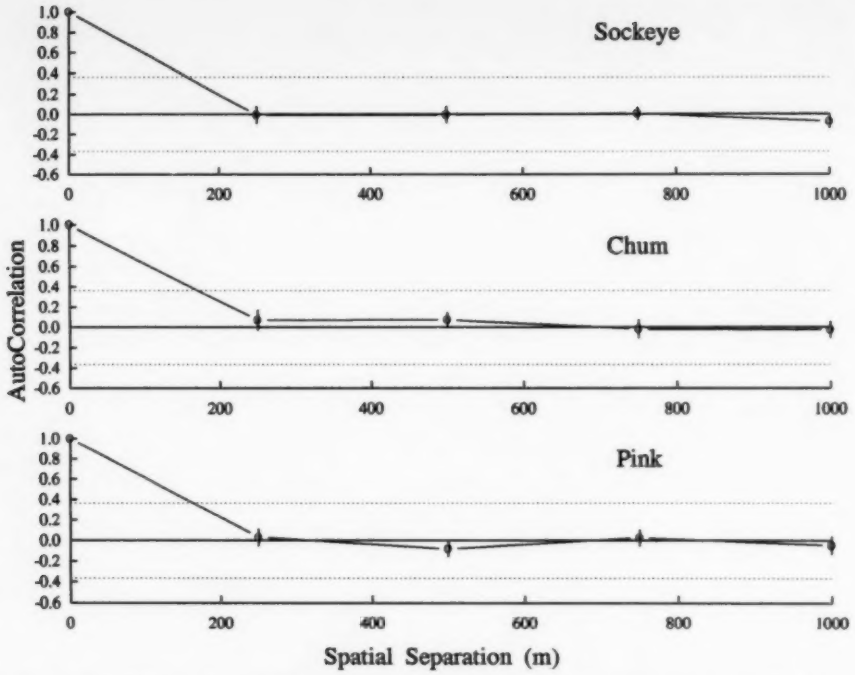

FIG. 2. Average spatial correlation between salmon catches in 5-tan (250-m) gillnet sections. Serial autocorrelation functions were calculated for catches from the 30 nightly catches and then averaged to calculate the mean serial autocorrelation ±2 SE. Horizontal lines indicate the approximate 95% confidence interval for an individual autocorrelation function.

TABLE 2. Properties of some relevant statistical distributions $f(x)$ and range for which $f(x)$ is valid. The algebraic definition of some definitions has been written in a nonstandard form in order to express the distribution in terms of the mean.

Distribution	$f(x)$	Parameters	Mean	Variance	Range
Normal	$\frac{1}{\sqrt{2\pi\sigma}} e^{\frac{-(x-\mu)^2}{2\sigma^2}}$	μ, σ	μ	σ^2	$(-\infty,\infty)$
Poisson	$\frac{\mu^x e^{-\mu}}{\Gamma(x+1)}$	μ	μ	$\sigma^2 = \mu$	$(0,\infty)$
Gamma	$\frac{k^x x^{k-1} e^{\frac{-k x}{\mu}}}{\mu^k \Gamma(k)}$	μ, k	μ	$\sigma^2 = \frac{\mu^2}{k}$	$(0,\infty)$
Negative binomial	$\frac{\Gamma(x+k)}{\Gamma(x+1)\,\Gamma(k)} \left(\frac{k}{\mu+k}\right)^k \left(\frac{\mu}{\mu+k}\right)^x$	μ, k	μ	$\sigma^2 = \mu + \frac{\mu^2}{k}$	$(0,\infty)$
Exponential	$\mu e^{-\mu x}$	μ	μ	$\sigma^2 = \mu^2$	$(0,\infty)$

latter case is more reliable, and the increased value for k defines exactly how much more reliable it is. Thus, each night's catch during the *Wakatake Maru* experiments is based on 20k df, because we have shown that the catch in each 5-tan net section is statistically independent.

Empirical Quantile–Quantile Plots

So far, we have shown that each 100-tan fishing operation is based on 20 or more df and that the variance increases at a rate intermediate between the Poisson and the gamma or exponential distributions. Quantile–quantile (QQ) plots are a nonparametric tool designed to evaluate the distributional form underlying a set of data (Chambers et al. 1983).

In forming a QQ plot, observations are sorted according to size and then the quantiles of the data are plotted against the corresponding quantiles of a set of random variables drawn from a specified statistical distribution. Departures from linearity are an indication that the distributional shape of the data does not closely follow that of the theoretical distribution, while differences in mean and variance cause changes in the location of the line and its slope, respectively.

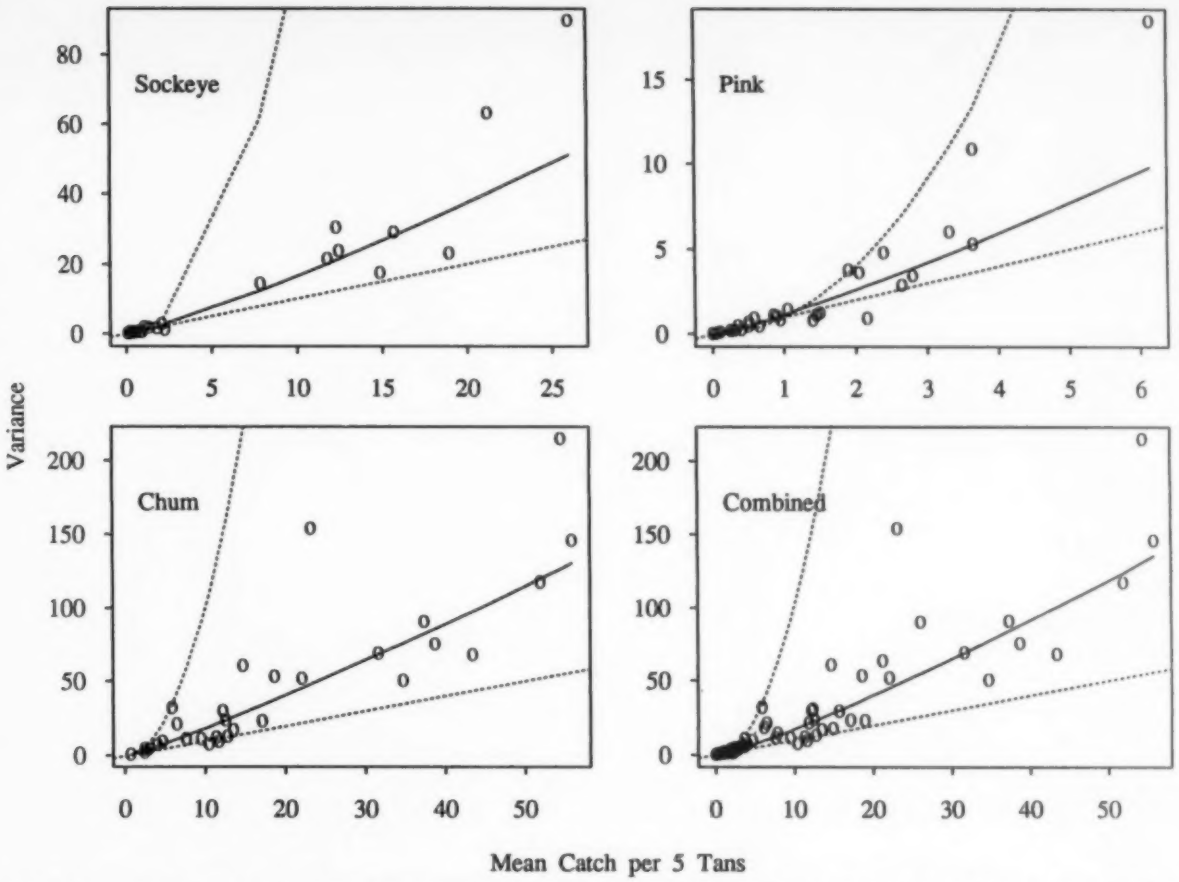

FIG. 3. Comparison of mean and variance of the salmon catch for each night's set along with the least-squares fit of the power curve (Table 1). Broken lines indicate the $\sigma^2 = \mu$ (Poisson) and $\sigma^2 = \mu^2$ (exponential, $k = 1$) reference lines.

A single QQ plot can therefore be used to assess all experiments because the distribution of the replicate samples should be correctly described by the mean μ_i for each of the $i = 1, 2, \ldots, 89$ experiments and (for the negative binomial and gamma distributions) the parameter k. If the a priori specification of distributional parameters is incorrect, then significant scatter will be evident.

QQ plots for the candidate distributions given in Table 2 were calculated using the statistical programming language S-Plus (Becker et al. 1988) (Fig. 4). Generation of random variables following the Poisson and negative binomial distributions was based on algorithms by Morgan (1984) and Rubinstein (1981), respectively. All three of the Poisson, negative binomial, and ganna distributions seem to fit the data very well where we have calculated the individual plots for the negative binomial and gamma distributions using the experimental means and assuming $k = 20$. However, as the mean to variance ratio for the Poisson and gamma distributions is inconsistent with that empirically observed, we therefore tentatively conclude that the negative binomial distribution is the best descriptor of the data.

Estimating k for Individual Sampling Units

As k increases, the negative binomial distribution approaches the Poisson, but for finite k the observed variance will always be larger than that for the Poisson. We calculated an estimate of the degrees of freedom available per 5-tan section of gill net and the approximate associated uncertainty by maximum likelihood, assuming that the catches are distributed according to the negative binomial distribution (Bliss and Fisher 1953). A comparison of these estimates suggests that over most of the range of salmon densities encountered, a constant k is consistent with the data (Fig. 5). However, for densities $\mu \leq 10$ (≤ 2 salmon/tan) the estimates of k are more erratic.

Large estimates of k are all associated with experiments where the sample variance was less than or equal to the mean. The variance can never be smaller than the mean for either the Poisson or the negative binomial distribution when $\mu \geq 1$, so the occurrence of some samples where this is true presumably reflects the effect of random sampling error at low densities (see Fig. 3). In these cases the estimate of k must become very large in order for the variance to approach the mean. Similarly, substantial scatter between the remaining individual estimates of k is evident. However, this scatter is not surprising, as the estimates of k for individual experiments are the best fits to frequency histograms that are based on only 20 samples.

An overall weighted moment estimator of k was developed by Bliss and Owen (1958) that can take into account all the information available from the 89 experiments. Applying this

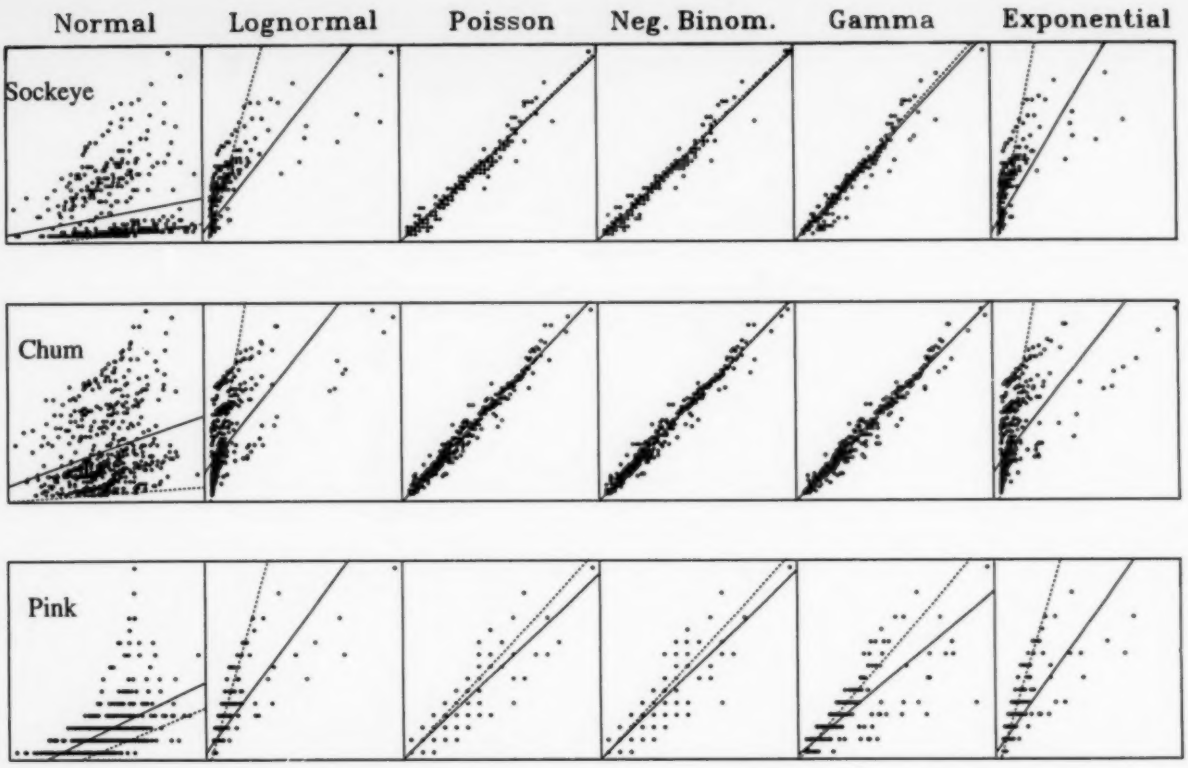

FIG. 4. Empirical quantile–quantile (QQ) plots. For the negative binomial and gamma distributions, $k = 20$. The 1:1 reference line is shown (broken line) along with the least-squares fit (solid line). A small amount of random noise was added to the plotted points to reduce overplotting.

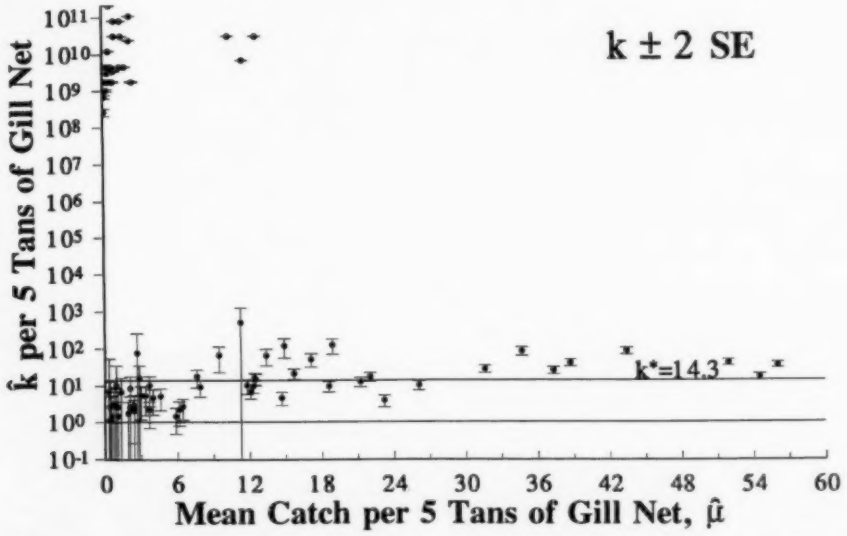

FIG. 5. Parameter estimates of k for the 89 experiments and approximate 95% confidence limits. Except at low salmon densities, there is little reason to reject the hypothesis of a single k for the negative binomial distribution.

estimator to the data for individual species yields a consistent estimate for chum and sockeye salmon of $k = 15.2$ and 15.9 df per 5-tan unit and a substantially smaller estimate of $k = 4.8$ df for pink salmon. The estimate of k for pink salmon is somewhat suspect, however, as mean pink salmon catches never exceeded $\mu = 6$ (Fig. 3). This places these catches in

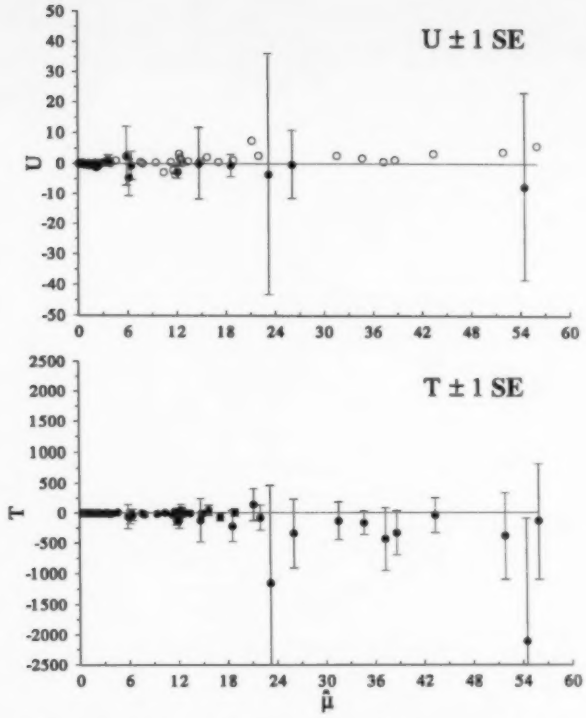

FIG. 6. Parametric tests of whether the sampling distribution for individual experiments is consistent with a negative binomial distribution. The tests compare the expected and observed second (U) and third (T) moments of the data, assuming the maximum likelihood estimates of k. The null hypothesis is that the statistic is zero for both tests. Open circles indicate point estimates of U for which approximate standard errors could not be calculated.

the range where rather erratic estimates of k are observed. An aggregate estimate of k, based on all 89 experiments treated simultaneously, leads to $k = 14.3$ df per 5-tan section (Fig. 5).

Testing the Fit of the Negative Binomial Distribution

These estimates of k assume that the underlying sampling statistics for the data follow a negative binomial distribution. Two parametric tests of the consistency of the negative binomial as a descriptor of the data are available, given an estimate of k. These tests compare the observed second and third moments of the data with those expected for a NB(μ_i, k_i) distribution (Bliss and Fisher 1953). The third moment test, T, is the more powerful test for our data (Evans 1953).

We calculated these statistics, and their approximate standard errors, using the maximum likelihood estimates of k_i for each of the 89 experiments. The results give little reason to reject the hypothesis that the variation in the observed data is consistent with that expected for samples drawn from a negative binomial distribution (Fig. 6). Recalculating these statistics using the aggregate estimate of $k = 15$ leads to the same conclusion, but estimates of U and T for individual experiments were more erratic than when the estimates of k were based on the individual maximum likelihood estimates of k, as expected.

Some further insight into why the individual estimates of k shown in Fig. 5 are more erratic at low mean densities can be obtained by comparing the shape of the negative binomial distribution as the sampling effort k increases. Typically, mean salmon catches on the high seas average roughly 3 salmon/tan, with very high catches sometimes approaching 10 salmon/tan. At high densities ($\mu = 10$ salmon/tan; Fig. 7), a k value equivalent to the estimate for the 100-tan commercial net ($k = 330$)

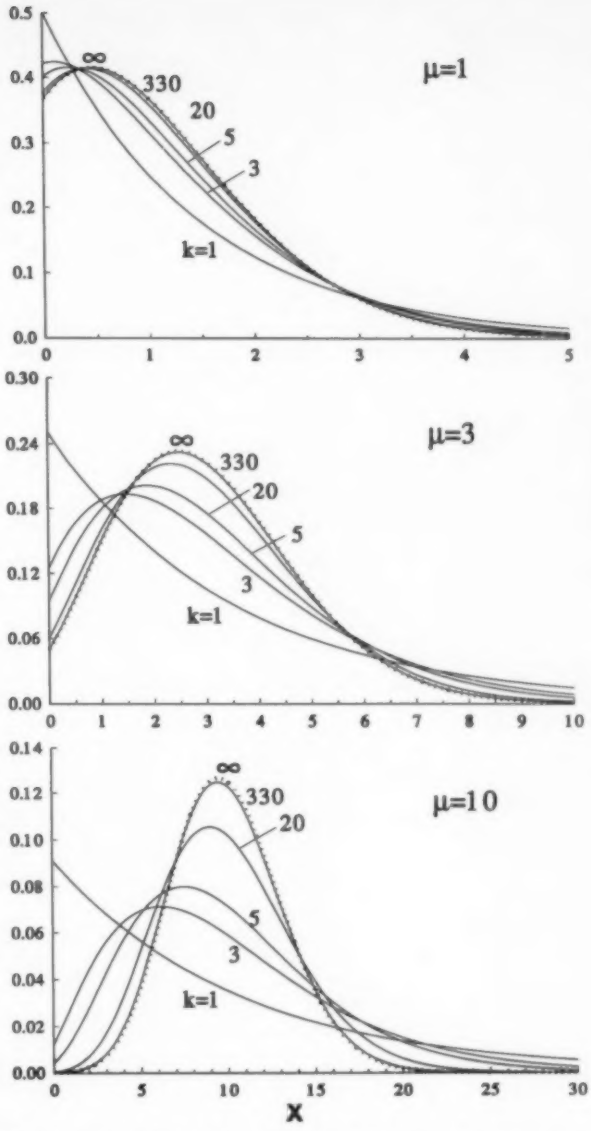

FIG. 7. Comparison of the sampling distribution of the negative binomial and Poisson distributions for three mean catch densities ($\mu = 1$, 3, and 10 salmon/tan). The shape of the negative binomial distribution is shown under the assumption that a single fishing operation provides only 1 df ($k = 1$), that each 250-m net section provides 1 df ($k = 20$), and that each commercial gill net provides approximately 330 df (= 5 km/15 m). The 3-df curve is the estimated sampling distribution for a single tan within the 100-tan commercial net. Note that even at high densities the negative binomial distribution is significantly skewed when the sampling effort k is low and that for a single independent observation the most likely catch is always zero. The Poisson distribution ($k = \infty$) is shown as a hatched line.

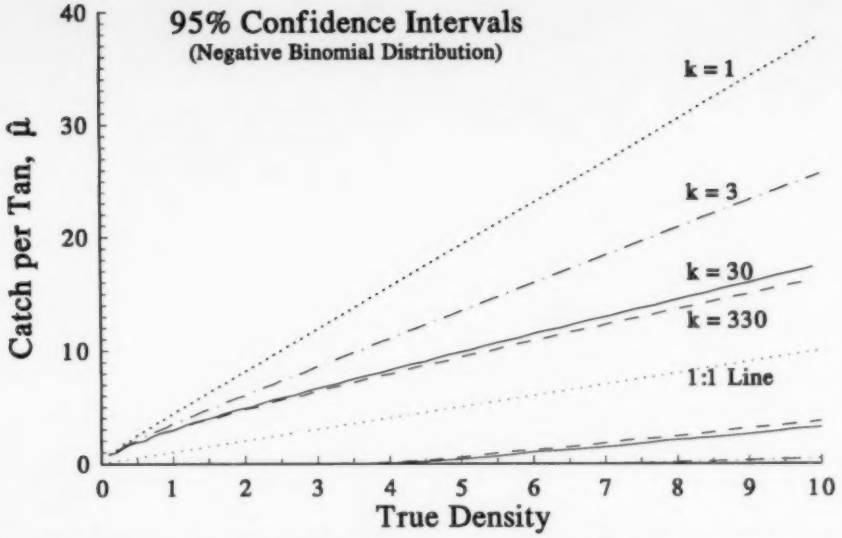

FIG. 8. Comparison of the width of the 95% confidence intervals at different sampling densities μ (numbers caught per tan) and different sampling efforts k. The graph is based on the negative binomial distribution, assuming equal probability densities in the tails. Little decrease in the width of the confidence interval is achieved by increasing sampling efforts beyond 10 tans ($k = 30$).

results in a sampling distribution that closely approaches the Poisson ($k = \infty$).

However, for lower degrees of freedom, appropriate if we assume only that 5-tan net sections are independent ($k = 20$), or that a single fishing operation provides only one observation on salmon density ($k = 1$), the shape of the distribution differs markedly from the Poisson. The distributional shape therefore depends sensitively on k at high salmon densities. In contrast, at low densities ($\mu = 1$), all distributions are highly skewed, and the shape of the negative binomial distribution changes little, irrespective of the sampling effort. Therefore, although the estimates of k at low densities are highly variable (Fig. 5), this variability does not have a marked influence on the shape of the frequency distribution.

An Application

Gill nets are widely used to sample salmon across the North Pacific. Japan has conducted the most extensive scientific sampling programs for salmon on the high seas since 1952, typically using 100–200 tans (5–10 km) of gill net per night at each sampling location. This data base forms an unparalleled source of information on the distribution and abundance of Pacific salmon on the high seas.

Concern with potential levels of bycatch, however, prompted the United States government to ban the use of drift nets within its 200-mile EEZ. Congress defined a drift net for legislative purposes as any gill net $\geq$1.5 miles in length. However, it is unclear to what degree reducing the length of gill net used would compromise the reliability of such surveys, or complicate the comparison of abundance estimates based on different net lengths.

Examining the effect of a reduced sampling effort is straightforward once the sampling distribution is established as negative binomial. We calculated the 95% confidence intervals by finding those estimates of μ below which 2.5 and 97.5% of the total sampling distribution occurs for a given catch density and sampling effort. The result (Fig. 8) shows that for a given density (catch in numbers per tan), increasing the sampling effort beyond approximately 10 tans ($k = 30$) gives relatively little decrease in the width of the 95% confidence interval. (The total catch does, of course, increase with the amouny of gear used.) As degrees of freedom are additive for the negative binomial distribution, this also implies that repeated sampling at the same location on different days will provide little additional information on local density and suggests that applying relatively small amounts of effort over a broader geographic region would be more informative.

Discussion

We draw two inferences from the work we have reported here. First, our results suggest that the catch statistics for Pacific salmon caught on the high seas can be satisfactorily approximated by the negative binomial distribution, with the shape of the underlying sampling distribution determined by the amount of fishing effort used and the density of the salmon. In practice, no statistical distribution can be an exact representation of real data, but we can determine whether the major features of the data can be described by some tractable statistical distribution. Under this criterion, the negative binomial appears to be a useful descriptor of the uncertainty inherent in high-seas gillnet sampling for salmon. This is a useful result because it allows us to directly estimate the nature of the variability associated with a single catch estimate without always requiring the use of replicate sampling.

Second, we have also demonstrated that a single observation of catch may carry with it substantially more than one degree of freedom, if sampling occurs on spatial scales exceeding the characteristic length scale of the organism being studied. This is probably the rule when large-scale sampling is carried out, as happens when commercial fishing gear is used. This result, which is particularly useful when the catch variation is consis-

tent with either the negative binomial or gamma distribution, allows us to precisely state how the uncertainty in a single estimate of catch-per-unit-effort varies with effort. To our knowledge, this is the first time that a clear statement of the effect of fishing effort on the statistical reliability of catch estimates has been made.

In operational terms, we have defined this characteristic length scale as the distance at which the correlation between spatially separated samples declines to zero. In part, this length scale is related to the ecology of the species, as different life history periods may be characterized by different levels of aggregation. The level of aggregation will affect the degree of spatial correlation present, and thus the distance that must be moved on average prior to obtaining an independent measure of population abundance. This phenomenon appears to be the reason why ecologists have argued that the "k" of the negative binomial distribution is an ecological constant that varies with species, life history, and habitat (Taylor 1961, 1984).

Some controversy surrounds the interpretation of k in ecology, with one view holding that a scaling law known as Taylor's Law, $\sigma^2 = \alpha\mu^\beta$, is of wide applicability (Taylor 1961, 1984) and that the ecological constant k of the negative binomial varies with species, life history, and habitat. Taylor's Law has been widely applied as a rule for transforming a set of data to constant variance and near-normality. However, the broad applicability of Taylor's Law has been challenged (Routledge and Swartz 1991).

Taylor (1984) in fact claimed that no biological justification for the parameter k of the negative binomial distribution has yet been given. However, our observation on the additivity of k, and its correspondence with the df on which a sample is based, does provide such a justification. We view the parameter k as a measure of the spatial length scale over which a species shows coherence, so that new degrees of freedom are only obtained when sampling occurs on length scales greater than this value. We justify this interpretation because the statistical distributions of sums of independent negative binomial or gamma samples belong to the same family, with additive degrees of freedom. Variation in estimates of k are therefore to be expected because the size of biological aggregations will vary with the life history stage.

However, the degrees of freedom k are also related to the size of the sampling gear relative to the size of the aggregation. A gill net twice as long, or a trawl sample covering twice the usual distance, obviously provides a more reliable estimate of population abundance. Providing a statistically rigorous description of this intuitive fact has, however, proven difficult. Fortunately, the degrees of freedom for two independent samples drawn from the negative binomial distribution are additive, so it is straightforward to establish the effect of differing levels of sampling effort on the statistical certainty of the result when the negative binomial (or gamma) distribution is an appropriate descriptor. This finding is analogous to the concept of the "effective degrees of freedom" achieved in time series analysis, where the autocorrelation present in a time series of animal abundances means that additional data points do not necessarily provide an additional full degree of freedom on the dynamics of the population unless the time between observations is long enough for the correlation to fall to zero (cf. Welch 1987a, 1987b).

One further reason for establishing the statistical distribution and degrees of freedom appropriate to a set of data is that it can lead to valuable physical insight into underlying processes, even when our observational unit (5 tans of gill net, or 250 m) is much larger than the relevant length scale. For example, the evidence that $k = 15$ df per 250 m of gill net allows us to infer that spatial coherence of salmon aggregations disappears on length scales on the order of approximately 250 m/15 df $\approx$ 15 m. This estimate is quite close to estimates of the size of salmon schools on the high seas that have been previously made, both by direct and indirect methods of observation (Mishima and Shimazaki 1969; and personal observations).

The negative binomial distribution can be generated under a number of assumptions (cf. Johnson and Kotz 1969). One way in which a negative binomial distribution arises naturally is as a result of a series of independent Poisson events occurring (e.g., salmon schools independently encountering a net), but with the size of each school varying according to a gamma probability distribution. The fit of the data to the negative binomial distribution therefore suggests that salmon probably form loose aggregations of varying sizes on the high seas at night, when the gill nets are deployed. However, the number of salmon in these aggregations is likely quite small, as both the mean catch and the estimated length scale for the spatial aggregations are low. Direct observations made on the high seas support this inference. Mishima and Shimazaki (1969) found that chum or pink salmon schools were most frequently composed of only two individuals and that the majority of schools consisted of six or less individuals. Schools of 12 or more salmon were not encountered.

The estimated length scale of salmon aggregations may increase during spawning migrations into coastal waters, when mature salmon probably form larger aggregations. If so, the sampling characteristics of the gear (and thus the shape of the negative binomial distribution) would differ because the effective number of observations that could be obtained from a given length of net would decrease. Some evidence for this can be found in early Japanese studies that suggest that significant levels of autocorrelation on length scales of 4 tans or less may be found during inshore spawning migrations in the Pacific Ocean, but not in the Okhotsk Sea (Mishima et al. 1965). If true, this would imply that an accurate description of the sampling distribution would require reestimating k in terms of the spatial length scale appropriate during the inshore migration phase of the Pacific salmon's life history.

Conclusions

Parametric statistical procedures allow powerful techniques to be applied to the analysis of large data sets, but the results depend on correctly specifying the sampling distribution from which the data are derived. Departures from specific assumptions can cause misleading results, such as when distributions with heavier tails than the normal or Poisson occur. For example, Poisson regression has recently been used in the analysis of fisheries survey data (e.g., Smith et al. 1991). However, if a heavier-tailed distribution such as the negative binomial is more appropriate, the uncertainty in the parameter estimates may be seriously underestimated, although the parameter estimates themselves may not be seriously affected (McCullagh and Nelder 1983; Moore and Tsiatis 1991).

Assuming an extra-Poisson variance for the model is a common technique used when there is evidence that the variance exceeds the mean (McCullagh and Nelder 1983). However, if the variance follows a power function of the mean, the statistical structure will still be in error, with insufficient weight

to observations at low densities and too much statistical weight given to observations made at high densities.

A priori identification of the sampling distribution can reduce some of these difficulties. Our results demonstrate that for the estimated degrees of freedom of the Japanese high-seas salmon commercial gill net, the sampling distribution closely approximates the Poisson over the range of catches typically encountered. However, at high densities the negative binomial distribution will be significantly more "heavy-tailed" than the Poisson, indicating that catches that would be considered "unusually" large or small according to the Poisson distribution could still occur relatively frequently. This could happen, for example, during coastal spawning migrations of salmon, when density is probably higher than on the high seas.

Data collected from fishing operations form an essential aspect of nearly all stock assessment procedures. It is therefore important that the sampling distribution and the degrees of freedom associated with each fishing operation be established as rigorously as possible, in order to correctly weight the information present in "unusually" large or small catches and to establish what level of sampling effort to use. When sampling from a negative binomial distribution the most likely catch is zero when the degrees of freedom are small, regardless of the true mean density. The presence of zero catches is a common occurrence in fisheries surveys (e.g., Pennington 1983, 1985, 1986; Mangel and Smith 1990) and therefore suggests that the negative binomial distribution may have broad applicability. However, because of the asymmetric nature of the negative binomial distribution at low densities or sampling efforts, it is essential to develop an accurate estimate of the degrees of freedom associated with the catch observations obtained with a given sampling gear. This requirement applies equally to any fishery where information from the catch is used to guide scientific studies or management actions.

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Use of Lanthanide Elements to Mass Mark Juvenile Salmonids

Bridget C. Ennevor and R.M. Beames

Department of Animal Science, University of British Columbia, Suite 248-2357 Main Mall, Vancouver, B.C., Canada V6T 1Z4

Ennevor, B.C., and R.M. Beames. 1993. Use of lanthanide elements to mass mark juvenile salmonids. Can. J. Fish. Aquat. Sci. 50: 1039–1044.

Two experiments were conducted to evaluate the mass marking of coho salmon (*Oncorhynchus kisutch*) fry and smolts by the addition of lanthanide acetates to the water supply. The results demonstrated that lanthanides are absorbed from the water and are subsequently deposited in the vertebral column, otoliths, and scales of juvenile coho. Lanthanum and samarium applied at concentrations of 100 μg/L for 6 wk to fry resulted in the retention of detectable levels in bony tissues after 10.5 mo. When coho fry and smolts were exposed to various combinations of lanthanum and cerium at a total concentration in the water of 100 μg/L over a 4-wk period, residual mean amounts of lanthanides in the vertebral columns were greater in smolts than in fry.

Deux expériences ont été réalisées pour évaluer le marquage de masse des alevins et smolts de saumon coho (*Oncorhynchus kisutch*) par l'addition d'acétates de lanthanide dans l'approvisionnement en eau. Les résultats ont montré que les lanthanides sont absorbés de l'eau et se déposent par la suite dans la colonne vertébrale, les otolithes et les écailles des saumons coho juvéniles. L'exposition d'alevins à du lanthane et à du samarium à des concentrations de 100 μg/L pendant 6 sem a entraîné la rétention de concentrations détectables de ces produits dans les tissus osseux après 10,5 mo. Lorsque alevins et smolts de coho ont été exposés à diverses combinaisons de lanthane et de cérium à une concentration totale dans l'eau de 100 μg/L pendant 4 sem, la quantité moyenne de lanthanides résiduels dans la colonne vertébrale était plus élevée chez les smolts que chez les alevins.

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The current method used in Canada and the United States for tagging juvenile salmon, viz. coded wire tags in combination with adipose fin clipping, is extremely labour intensive with regard to both application and recovery of tags. A technique that could greatly increase numbers marked and eliminate handling of individual fish would be a great asset to fisheries management and hatchery assessment programs. Chemical marking offers an alternative, but any chemical must satisfy certain criteria: that it not be harmful, not cause unusual behaviour patterns, have no harmful effects on metabolism, can be applied quickly and easily without handling the fish, is inexpensive to apply, and differences between marked and unmarked populations are not distinguishable by predators (Trefethen and Novotny 1961). Another important consideration is the safety of the chemical in fish destined for human consumption. The disadvantage of chemical markers is that, if used alone, the marked fish cannot be visually identified.

Strontium, applied through the diet and the water supply, has been used as a marker with no apparent adverse effects on the fish (Behrens Yamada et al. 1979; Behrens Yamada and Mulligan 1982; Behrens Yamada and Mulligan 1987; Guillou and de la Noüe 1987). However, the abundance of strontium in seawater dictates that, for a migrating marine species, a method is needed to differentiate elemental concentrations in the various concentric growth rings of scales. Such a technique is laser ablation by inductively coupled plasma–mass spectrometry (ICP-MS) which involves a series of successive laser shots to identify marked rings on the scales. Unfortunately, this technology is still in the developmental stages and tends to be expensive due to the extensive use of the laser required for spatial differentiation.

The lanthanide elements (rare earths) have many characteristics that make them suitable for marking fish. They do not occur naturally in salmon; also, they are bone-seeking (Durbin et al. 1956; Jowsey et al. 1958), nonradioactive (except for promethium), and safe to handle. Because the bony tissues of fish are deposited concentrically (Norris et al. 1963), and the elements contained therein are not recycled as in mammals (Ham 1952), the biological half-life of the lanthanide elements in these tissues is long.

Although there has been considerable work done on the toxicity of lanthanides in mammals, none has been done specifically on fish. When rats were fed oral doses of samarium and gadolinium of up to 2 g/kg body weight, there were no mortalities or evidence of internal damage (Haley et al. 1961). When a range of levels have been administered to mammals, toxicity generally has decreased with increasing atomic weight (Kyker and Cress 1957; Luckey and Venugopal 1977).

There have been only a few experiments that have explored the possibility of using the lanthanide elements to mark fish. Europium provided in the feed at concentrations of 1500 μg/g (Anonymous 1974) has been detected in the scales 1 yr after labelling. However, the water treatments using dysprosium employed by Miller (1963) and Babb et al. (1967) were ineffective because of the short exposure times that were used and the low treatment concentrations. Samarium administered by intraperitoneal injection and by addition to the diet was tested by Michibata (1981) and Michibata and Hori (1981). These workers postulated that, with treated food, the absorption was actually from the water as a result of food and faecal contamination. This contention is supported by the fact that, in other animal species, lanthanide elements have been shown not to be

absorbed in the intestinal tract (Kyker 1961; Ellis 1968; Luckey and Venugopal 1977). These results suggested that a promising alternative for marking fish with these elements was to add them to the water supply.

Consequently, after a series of preliminary investigations, two experiments were conducted using coho salmon (*Oncorhynchus kisutch*) fry and smolts. In the preliminary work, coho and steelhead (*Oncorhynchus mykiss*) alevins were shown to be too sensitive to the lanthanides for this stage to be of any use for marking (Ennevor 1991). Coho fry were then tested. When these were exposed to lanthanum at a concentration of 100 μg/L for 3 wk, detectable levels were accumulated in vertebral columns, otoliths, and scales. A suitable method of administration was found to be by addition of a constant proportion of a concentrated lanthanide acetate solution to a constant flow of ambient river water. This allowed for a continual replenishment of the elements absorbed by the fish and maintained a suitable water quality; also, it was compatible with current hatchery procedures.

The purpose of the first experiment was to determine toxic effects and levels of accumulation of the lanthanides in the vertebral column, otoliths, and scales for up to 10.5 mo posttreatment. Coho fry were exposed to two lanthanides at concentrations of 10 or 100 μg/L in the water supply for two durations (3 or 6 wk). The elements selected were of different weight classifications: light weight (lanthanum) and medium weight (samarium). For marking with lanthanides to be of practical value, two or more lanthanides would have to be used in various combinations in order to be able to differentiate stock from several hatcheries. Thus, the second experiment was designed to test effects of simultaneous exposure of two lanthanides. The effect of life cycle stage was also investigated. The design involved the administration of various combinations of two light-weight lanthanides, lanthanum and cerium, to coho fry and smolts.

Methods

All experimental fish were exposed to ambient river water to which concentrated aqueous solutions of lanthanide acetates had been added. After the completion of the labelling, bony tissues were extracted and analysed for lanthanide accumulation.

Chemicals Used

All lanthanides used were in the trivalent acetate salt form. These salts, regardless of degree of hydration before addition, convert to the hydrated form at low concentrations and dissolve readily in water (Topp 1965). Lanthanides (and suppliers) were yttrium acetate tetrahydrate and cerium acetate (Alfa Catalog Chemicals, Davers) and lanthanum acetate hydrate, samarium acetate hydrate, dysprosium acetate hydrate, and ytterbium acetate tetrahydrate (Aldrich Chemical Company Inc., Milwaukee).

A 6% sodium hypochlorite solution (BDH Chemicals Inc., Toronto) was used to digest the traces of tissue from the vertebral column and otoliths. This solution was analysed by ICP-MS and found to have a total lanthanide content of less than 2 μg/L.

Water Samples

Water samples were taken at the beginning and end of both experiments and at weekly intervals. Concentrated nitric acid was added to the samples (0.5 mL acid/10 mL water) to minimize adherence of these elements to the container surfaces. The samples were then sealed and stored at 4°C until analysed.

Vertebral Column Extraction

After the withholding of feed for 24 h the fish were asphyxiated and weighed. The heads were then removed and frozen for otolith removal at a later date. The vertebral columns were dissected out and the majority of the flesh removed. The remainder of the flesh was removed with 6% sodium hypochlorite solution with careful monitoring to ensure that all the flesh was dissolved, but none of the bony tissue. The vertebral columns were then rinsed in distilled water, dried overnight at 70°C, and weighed.

Otolith Extraction

For removal of the otoliths, the entire fish head was placed in a petri dish with 6% sodium hypochlorite solution. As the otoliths are the densest structures in the fish body and are composed of calcium carbonate, they are able to withstand the digestion procedure (Treacy and Crawford 1981). After overnight digestion, only the otoliths remained in the dish. The sagittae were removed and rinsed in distilled water, placed in a vial, and dried overnight.

Scale Samples

Scales were removed from the area between the lateral line and the dorsal fin just posterior to the dorsal fin. They were then soaked for 24 h in distilled water to remove mucus, and the cleanest nonregenerated scales were chosen for analysis.

Lanthanide Determinations

Tissue and water samples were analysed for lanthanide content using ICP-MS by Elemental Research Inc., North Vancouver. Prior to analysis, the tissue samples were digested in nitric acid and then diluted with distilled water. The results for all tissues were reported as a concentration relative to the dry sample weight, with the concentrations of the otolith and scale samples being calculated relative to calcium concentration, which was set at 10%. The water samples were analysed without modification.

Statistical Analyses

Experiments 1 and 2, which incorporated factorial designs, were analysed by analysis of variance using SYSTAT (Wilkinson 1989), with differences between means tested at $P \leq 0.05$ using Tukey's multiple range test. In both experiments, because the untreated fish had no detectable lanthanide content and consequently no variance, the results from these tanks were not included in the statistical analyses. Data from replicate tanks were pooled, since tank effects were not significant. An experimental error required that data for lanthanum and samarium treatments in Experiment 1 be analysed separately.

Experimental

Experiment 1

The purpose of this experiment was to investigate limits of toxicity and levels of accumulation and retention of lanthanum

TABLE 1. Treatment combinations of lanthanum (La) and cerium (Ce) used in Experiment 2.

Week	Treatment 1, fry and smolts	Treatment 2, fry only	Treatment 3, fry only
1	La, 100 μg/L	La and Ce, 50 μg/L	No element
2	La, 100 μg/L	La and Ce, 50 μg/L	La and Ce, 100 μg/L
3	Ce, 100 μg/L	La and Ce, 50 μg/L	La and Ce, 100 μg/L
4	Ce, 100 μg/L	La and Ce, 50 μg/L	No element

and samarium in various bony tissues of labelled fry. The design involved a factorial arrangement with five lanthanide treatments (control with no lanthanide, 10 μg lanthanum/L, 100 μg lanthanum/L, 10 μg samarium/L, and 100 μg samarium/L), two durations of exposure (3 and 6 wk), and six sampling times (2 wk after termination of treatments and thereafter every 2 mo). There were two replicates of each treatment, except for the samarium treatments. Due to an experimental error, one replicate of the 10 μg samarium/L treatments and one replicate of the 100 μg samarium/L treatments were inadvertently reversed 10 d after the start of the experiment. Since this changed the exposure regime, the results from fish in these tanks were not used in the statistical analysis.

Capilano hatchery brood stock coho fry were removed from incubation trays approximately 3 mo prior to the start of the treatments. At the start of the experiment the mean weight of the fry was 6 g. The fry were fed Oregon Moist Pellets (OMP) twice daily for the duration of the experiment. Ambient water temperature increased steadily from 7 to 10°C throughout the labelling period. The river water and the diet contained undetectable levels of lanthanides.

At the termination of the lanthanide exposures, the fry were placed in segregated minitroughs and maintained for 10.5 mo. Five fry were sampled from each trough 2 wk after treatments ended and then every 2 mo. Vertebral columns were removed at all six sampling times, otoliths were removed at the initial and final sampling times, and scales were removed only at the final sampling time.

Experiment 2

This experiment was designed to investigate the simultaneous incorporation of two lanthanides in coho fry and smolts, with measurements taken on retention and toxicity. The design involved a factorial arrangement, consisting of two stages of development (fry and smolts) and four treatment combinations of lanthanum and cerium (three treatments plus negative control), with two replicates and two missing blocks. Thirty coho fry (mean weight 0.5 g) were placed in each of eight tanks and 15 coho smolts (mean weight 19.4 g) were placed in each of four tanks. Duration of treatments was 4 wk (Table 1).

The water temperature ranged from 4.5 to 6.5°C throughout the labelling period. The fish were fed OMP daily for the duration of the experiment. After the 4-wk treatment period, there was a 2-wk rinse period during which the fish were provided with untreated river water. At the end of the rinse period, vertebral columns and otoliths were removed from five fish in each tank.

Results

Experiment 1

At similar concentrations, lanthanum was more toxic than samarium (Table 2). The fry were not sensitive to lanthanum at 10 μg/L or to samarium at 10 or 100 μg/L. There were significantly more mortalities during the 6-wk treatment period in the tanks containing lanthanum at a concentration of 100 μg/L than in all other tanks. Throughout the 10.5-mo growth period after treatment, average mortality was less than 0.05%, with no clear treatment effect.

TABLE 2. Coho fry mortalities during the treatment periods of Experiment 1 (lanthanum (La) and samarium (Sm) treatments) and Experiment 2 (lanthanum and cerium (Ce) treatments). Within experiments, values without a common letter are significantly ($P \leq 0.05$) different according to Tukey's test.

Treatment	Mortalities (%)	
Experiment 1	3 wk	6 wk
Control	1 a	1 a
Sm, 10 μg/L[a]	0 a	1 a
Sm, 100 μg/L[a]	0 a	0 a
La, 10 μg/L	1 a	1 a
La, 100 μg/L	4 b	11 b
Experiment 2	4 wk	
Control	1 a	
La, 100 μg/L and then Ce, 100 μg/L	0 a	
La, 50 μg/L and Ce, 50 μg/L	1 a	
La, 100 μg/L and Ce, 100 μg/L	12 b	

[a]Only one replicate used due to treatment reversal (see text for explanation).

Growth rate of all groups of fry was low throughout the 6-wk treatment period and the 10.5-mo growth period, with no significant differences in growth rate between treated and nontreated groups. The average weight of the fry was 3.6 g at the first sampling at 2 wk, rising to 7.1 g at the 2.5-mo sampling, and thereafter nonsignificantly to 9.0 g at the 10.5-mo sampling.

Lanthanum was absorbed more efficiently than samarium (Tables 3 and 4). For all sampling times, there were significantly greater amounts of element in vertebrae, otoliths, and scales of fry treated with lanthanum or samarium at concentrations of 100 μg/L for 6 wk than of all other groups. The amount of lanthanum or samarium in the vertebral columns remained relatively constant throughout the 10.5-mo growth period, although the concentration decreased as would be expected. For both elements, the concentration in the otoliths decreased to much lower levels (0.4 μg/g for lanthanum and 0.1 μg/g for samarium) than in the other tissues over the 10.5-mo period. At the 10.5-mo sampling, greater concentrations of both lanthanum and samarium were found in the scales than in vertebrae and otoliths (Fig. 1).

Experiment 2

Mortalities occurred only in the fry, and then only in the tanks containing lanthanum and cerium, each with concentra-

TABLE 3. Mean amount (ng) of lanthanum in vertebral columns of fry labelled with lanthanum showing the effects of lanthanum treatment concentration, duration of lanthanum exposures, and sampling time in Experiment 1. UD = undetectable amounts; overall mean lanthanum amount = 6.25 ng; error mean square = 7.369; total observations (n) = 240. Within comparison groups (21 individual treatment means, four duration × concentration means, and six sampling time means), mean values without a common letter are significantly ($P \leq 0.05$) different according to Tukey's test.

	Treatment and duration				
	10 μg/L		100 μg/L		
Sample time (mo)	3 wk	6 wk	3 wk	6 wk	Mean
0.5	1.35 a	2.68 ab	7.81 cd	12.23 d	6.01 a
2.5	0.90 a	3.84 abc	4.80 abc	17.17 f	6.68 a
4.5	2.00 a	4.33 abc	6.81 bc	17.24 f	7.59 a
6.5	UD	4.48 abc	4.02 abc	13.40 ef	7.30 a
8.5	UD	2.68 ab	4.96 abc	12.11 de	6.58 a
10.5	UD	2.88 ab	4.19 abc	11.83 de	6.30 a
Mean	1.41 a	3.48 b	5.43 c	14.00 d	

TABLE 4. Mean amount (ng) of samarium in vertebral columns of fry labelled with samarium showing the effects of samarium treatment concentration, duration of samarium exposures, and sampling time in Experiment 1. UD = undetectable amounts; overall mean samarium amount = 3.44 ng; error mean square = 2.894; total observations (n) = 120. Within comparison groups (21 individual treatment means, four duration × concentration means, and six sampling time means), mean values without a common letter are significantly ($P \leq 0.05$) different according to Tukey's test.

	Treatment and duration				
	10 μg/L		100 μg/L		
Sample time (mo)	3 wk	6 wk	3 wk	6 wk	Mean
0.5	0.31 a	1.43 ab	2.60 ab	8.73 cd	3.27 a
2.5	0.00 a	1.55 ab	2.00 ab	11.20 d	3.69 a
4.5	1.23 ab	0.72 ab	4.92 bc	11.34 d	4.55 a
6.5	UD	0.76 ab	1.19 ab	10.41 d	4.12 a
8.5	UD	0.71 ab	2.51 ab	7.70 cd	3.64 a
10.5	UD	0.86 ab	2.64 ab	7.26 cd	3.59 a
Mean	0.52 a	1.01 a	2.64 b	9.44 b	

TABLE 5. Mean lanthanide concentrations (μg/g) in otoliths of fry and smolts labelled with lanthanum (La) and cerium (Ce) showing the significant effects of lanthanum and cerium treatments in Experiment 2 (for explanation of treatments, see Table 1). n/a = missing blocks; overall mean lanthanide concentration = 0.11 μg/g; error mean square = 0.004; total observations (n) = 32. Within comparison groups (eight treatment × lanthanide × stage, four lanthanide × stage), mean values without a common letter are significantly ($P \leq 0.05$) different according to Tukey's test.

	Fry		Smolts	
Treatment	La	Ce	La	Ce
1	0.14 b	0.22 a	0.02 b	0.05 b
2	0.10 b	0.14 b	n/a	n/a
3	0.10 b	0.12 b	n/a	n/a
Mean	0.11 ab	0.16 a	0.02 b	0.05 b

tions of 100 μg/L applied for 2 wk (Table 2). For the labelled coho smolts, level of mortality was relatively low (0.5%).

At the time of tissue sampling (2 wk after the conclusion of the 4-wk experiment), there were significantly greater amounts of both lanthanum and cerium in the vertebral columns of smolts (mean 8.9 ng) than in the vertebral columns of fry (mean 1.5 ng), although mean concentration in the vertebral columns of fry (0.48 μg/g) was higher than in the vertebral columns of smolts (0.10 μg/g). There was no significant difference in the level of accumulation between lanthanum and cerium. Within stage of growth, treatment effects were not significant for either tissue lanthanide concentration or amount stored in the vertebral columns.

The concentration of cerium in the otoliths of the fry labelled with lanthanum at 100 μg/L for 2 wk and then with cerium at 100 μg/L for 2 wk was significantly greater than the concentration of lanthanum or cerium for all other treatments (Table 5). Mean lanthanide concentration in otoliths of fry (0.14 μg/g) was significantly greater than mean concentration in otoliths of smolts (0.04 μg/g).

Discussion

Juvenile coho salmon were successfully labelled with lanthanum, samarium, and cerium by addition of these elements to the water supply. In Experiment 1, lanthanum and samarium were found to be present in the vertebral column, otoliths, and scales 10.5 mo posttreatment. The amount of element remained

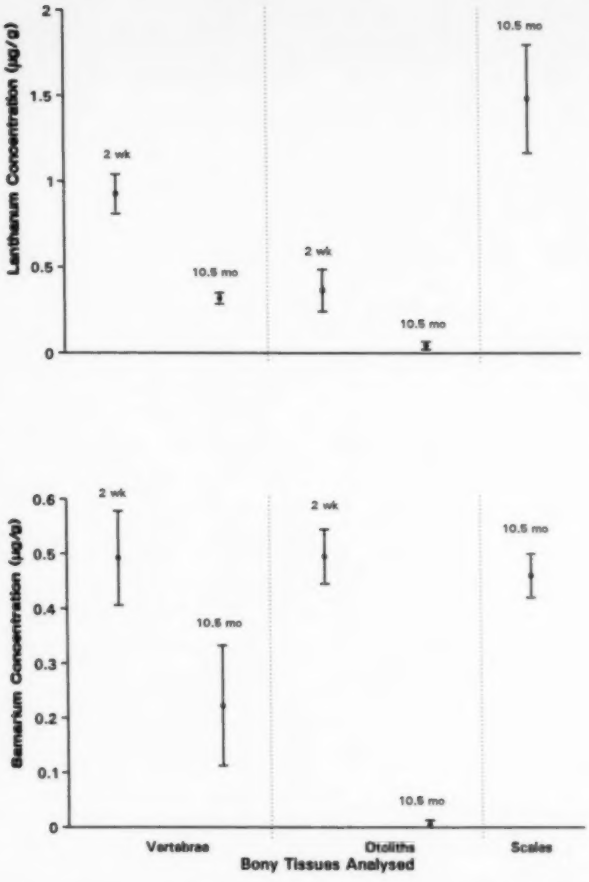

FIG. 1. Concentration of lanthanum or samarium present in the bony tissues of coho fry 2 wk and 10.5 mo after termination of the 6-wk treatment with 100 µg lanthanum or samarium/L in Experiment 1. Results reported as mean ±2 SE in µg/g dry tissue.

relatively constant in the vertebral column throughout this period, suggesting the likelihood of retention persisting through to adult fish. In these experiments the vertebral column was preferred as the sampling tissue because of its large size relative to scales and otoliths. Although this was an important consideration in this work where the fish were small, scales would appear to be more suitable for the sampling of returning adults. The scales would have many advantages, including a higher lanthanide concentration, ease of sampling, and elimination of the need to sacrifice the fish.

Experiment 2 demonstrated the feasibility of incorporating multielement marks in coho fry and smolts. This experiment had shown that a more effective mark can be laid down in smolts than in fry. This was most likely due to the greater daily growth increment of larger fish. This demonstrated the advantage of marking fish at the largest size which is compatible with hatchery practices.

In Experiment 1, the lighter element, lanthanum, was more toxic and was more efficiently absorbed than samarium. This finding agrees with those of Kyker and Cress (1957) and Luckey and Venugopal (1977) with mammals. In Experiment 2, where two light lanthanides were used in combination, it was found that the toxic effects were additive, since the highest mortalities were in tanks containing both elements simultaneously. Consequently, if two or more lanthanides were to be used simultaneously, a reduction in the concentration of each lanthanide would be necessary.

A major concern with the marking of hatchery-production fish is the ability to lay down a mark without seriously affecting survival or growth. Fish were weighed throughout the 10.5-mo growth period in Experiment 1, but growth rate was abnormally low for both treated and nontreated groups. A possible explanation for this may have been the small number of fish maintained in each 35-L container. Unfortunately, our results were unable to provide any information regarding this important question, since any possible adverse effects of the lanthanides on growth may have been masked by this overall slow growth.

Of potential concern is the possible inclusion of vertebral columns and other bones labelled with lanthanides in salmon that are canned for human consumption. However, any lanthanides contained in such products would not be absorbed by the human gastrointestinal tract (Cochran et al. 1950; Haley 1965; Luckey and Venugopal 1977). Environmental contamination is another possible concern. In the first experiment, river water samples were collected 10 m to 3.5 km downstream from the discharge. Lanthanum was detected at 0.09 µg/L at 10 m and 3.5 km, while samarium concentations were <0.03 µg/L at all distances. These levels, which are at the limits of detection by ICP-MS, are comparable with the levels in the water at the hatchery intake. Dilution of effluent resulting in extremely low residual concentrations should nullify the potential problems of toxicity and of the labelling of fish downstream.

ICP-MS was the method of analysis used to detect these elements because of its markedly lower detection limit than that of other known methods (Longerich et al. 1987; Houk and Thompson 1988). Electrothermal vapourization and laser ablation used in association with ICP-MS will allow for an increased sensitivity and even lower detection limits. Since spatial differentiation is not required for detection of extremely low concentrations of lanthanides in scales of returning adults, a single laser shot should be sufficient and cost effective for analysis.

Both the natural elemental abundance and the analytical properties of each of the lanthanides need to be considered. The elements with high abundance of a single isotope with little or no interference from other isotopes will be preferable for analysis by ICP-MS. Since many of the lanthanides are quite abundant and are easy to purify (Taylor 1964; Topp 1965; Kilbourn 1988), they are relatively inexpensive. The price (1990) of the lanthanide acetates varied from $18/100 g for lanthanum acetate hydrate to $170/100 g for ytterbium acetate tetrahydrate. Taking these factors into account, the lanthanides that appear to be feasible for use on a commercial basis for mass marking entire hatchery populations include lanthanum, cerium, praseodymium, neodymium, samarium, gadolinium, terbium, dysprosium, holmium, ytterbium, and yttrium (the last is not a lanthanide, but has similar properties).

In summary, these experiments have shown that the lanthanide elements can be added to the water supply at the fry and smolt stages to deposit a long-lasting mark in the bony tissues. Both coho fry and smolts were able to tolerate a concentration of 100 µg/L in the water supply. However, before the technique can be fully utilized on a large scale, several areas need to be explored, including the testing of the suitability of a wider range of lanthanides and the determination of the

amount of lanthanide that needs to be deposited for identification of mature adults. The effects of lanthanide administration on histopathology and immunocompetence should also be investigated.

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Spatial Variations in Nutrient and Particulate Matter Concentrations in Pyramid Lake, Nevada, USA, during a Dry Period

Martin E. Lebo, John E. Reuter, Charles R. Goldman, and Cathryn L. Rhodes

Division of Environmental Studies, University of California, Davis, CA 95616, USA

and Nancy Vucinich and Dan Mosely

Pyramid Lake Fisheries, Star Route, Sutcliffe, NV 89510, USA

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Spatial heterogeneity of nutrients, particulate matter, and chlorophyll were examined in Pyramid Lake during a dry period. In May 1991, variations in constituent concentrations were associated with shallow regions (chlorophyll), upwelling regions (nitrate), and river inflow (silicate and total P). However, only the spatial pattern of elevated silicate concentration near the mouth of the Truckee River was consistent throughout the year. Late-spring chlorophyll enrichment in shallow regions like the south basin appeared to be part of seasonal variations between the deep, central region of the lake and shallows possibly associated with light availability. During the study period, processes associated with strong winds appeared to be an important source of spatial heterogeneity. Profiles and spatial distributions of temperature and nitrate concentration during May 1991 indicated upwelling along the northwest and southwest margins of the lake. Examination of wind data for the region revealed that winds favorable for upwelling were common at Pyramid Lake, and temperature profiles along the north–south axis of the lake indicated additional upwelling events in June and September 1990. Strong winds also transport a large quantity of dust over the lake surface, which may be a major source of particle-bound P.

L'hétérogénéité spatiale des éléments nutritifs, des matières particulaires et de la chlorophylle a été examinée dans le lac Pyramid au cours d'une période sèche. En mai 1991, les variations de la concentration des constituants ont été liées aux régions peu profondes (chlorophylle), au région de remontée d'eau (nitrate) et aux embouchure des rivières (silicate et P total). Toutefois, seule la répartition spatiale de la concentration élevée de silicate près de l'embouchure de la rivière Truckee a été uniforme durant toute l'année. L'enrichissement en chlorophylle en fin de printemps dans les régions peu profondes comme le bassin sud a semblé faire partie des variations saisonnières entre la région centrale profonde du lac et les régions peu profondes, et pourrait être lié à la disponibilité de la lumière. Au cours de la période d'étude, les processus faisant intervenir des vents forts ont semblé être une source importante d'hétérogénéité spatiale. Les profils et la distribution spatiale de la température et de la concentration de nitrate au cours du mois de mai 1991 ont indiqué une remontée d'eau le long des bords nord-ouest et sud-ouest du lac. L'examen des données sur les vents de la région a révélé que les vents favorables au phénomènes de remontée d'eau étaient fréquents dans la région du lac Pyramid et les profils de température le long de l'axe nord–sud du lac ont indiqué des événements de remontée d'eau additionnels en juin et septembre 1990. Les vents forts transportent également une grande quantité de poussière à la surface du lac, ce qui pourrait être une importante source de P lié à des particules.

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Spatial variations in nutrient and particulate organic matter (POM) concentrations in lakes are the result of the nature and location of sources, internal processing, and physical forcing mechanisms (Goldman and Horne 1983). In large lakes, generally defined as systems where the pelagic zone is the dominant habitat (Imboden 1990), physical processes associated with winds have a major impact on biological production (Goldman and Jassby 1990; Tilzer 1990). Wind-driven processes in large lakes lead to a deeper surface mixed layer compared with small lakes (Patalas 1984) and the upwelling of nutrient-rich hypolimnetic waters along lake margins (Mortimer 1963; Neilson and Stevens 1987; Simons and Schertzer 1987; Strub and Powell 1987). In addition to driving mass transport of surface waters, winds also affect the distribution of plankton (Galat and Verdin 1988; Webster 1990).

Nutrients and POM entering lakes in river inflow also create heterogeneity, especially when fluvial concentrations differ from those in lake water. Generally, nutrient and POM concentrations are sufficiently high in running waters to act as a source of nutrients and POM (Goldman and Horne 1983). However, when the concentration of a constituent is lower in river inflow than in bulk lake water, the concentration in the lake should be diluted. In saline lakes, river inflow can also affect mixing of the water column through dilution of salty surface waters (Galat and Verdin 1988; Lebo et al. 1992).

Processes that affect biological production in lakes can also contribute to spatial heterogeneity by creating regions or "patches" of higher production relative to bulk lake water. For example, the warming and stratification of inshore waters of Lakes Ontario and Huron during spring lead to enriched

chlorophyll and POM concentrations in these regions, while offshore waters remain unstratified (Davis and Schelske 1976; Neilson and Stevens 1987). Spatial differences in nutrient inputs to surface waters during stratified periods could also contribute to variability in production. Because nutrients are efficiently transferred from surface to deep waters in large lakes through the settling of plankton (Tilzer 1990), the resupply rate may be an important factor regulating nutrient availability in surface waters and hence productivity. Bottom depth should affect the rate of nutrient transfer. In Pyramid Lake, Galat (1986) reported that phytoplankton productivity during summer may be higher in the shallower (<60 m), southern basin compared with the main body of the lake.

In this paper, we examine the spatial distributions of nutrients and POM in Pyramid lake, Nevada, USA, during 1989–91, a period with low river inflow. Using synoptic data collected in May 1991, spatial differences between the northern and southern basins of the lake and between shallow and deep stations are investigated. We determine how important the spatial trends observed for May 1991 are on a seasonal basis by comparing temporal data collected from index stations located in each basin. Finally, we briefly contrast the above description of spatial variations in nutrients and POM during this dry period with data collected during 1983–84 and 1986 to place the present observations in the context of the overall ecology of Pyramid Lake.

Study Site

Pyramid Lake is a large (450 km^2), terminal, hyposaline lake in western Nevada with a salinity of 4.3 ppt. The only permanent source of surface runoff to the lake, the Truckee River, arises in the Sierra Nevada moutains of eastern California and western Nevada. In transit, the Truckee passes through the city of Reno, Nevada, and downstream of Reno, transbasin agricultural water diversion claims approximatley 42% of total river flow (Milne 1987). This diversion of water out of the watershed has led to a 30% decrease in lake volume since 1905 (Galat et al. 1981). The lake is 40 km long and its width varies from 6.5 km at the south end to 16 km in the north. Pyramid Lake is a graben lake and is the deepest saline lake in the Western Hemisphere (Horne and Galat 1985). At present, maximum and average depths are 102 and 60 m, respectively.

Pyramid Lake is a warm, monomictic lake, although temporary meromixis can occur during periods of high Truckee River inflow (Galat and Verdin 1988). The lake mixes during winter (January–February) and is thermally stratified at 20–25 m between June and October (Galat et al. 1981; Lebo et al. 1992). The lake is highly alkaline (23 meq·L^{-1}, pH = 9.1–9.3), and its predominant ions are Na^+, Cl^-, and HCO_3^--CO_3^{2-} (Galat et al. 1981). At all times, Pyramid Lake is supersaturated with aragonite (Galat and Jacobsen 1985), and precipitation of $CaCO_3$ around surface springs is common.

As a desert lake, Pyramid is P rich and N deficient (Hutchinson 1937; Galat et al. 1981) with a dissolved inorganic elemental N:P ratio generally <2 (Reuter et al. 1993). The shores of the lake are sparsely vegetated and mountainous, and the lake is subject to intense dust storms primarily during spring (Galat et al. 1981). When compared with other saline lakes, Pyramid is oligotrophic with a mean chlorophyll concentration of 3.3 μg·L^{-1} (Galat et al. 1981), although maximum volume-weighted mean chlorophyll in surface waters during 1990 was only 2.7 μg·L^{-1} (Lebo et al. 1992). The phytoplankton assemblage is generally dominated by diatoms during the early spring, diatoms and green algae during summer, and blooms of *Nodularia spumigena* during summer–fall in certain years (Galat and Verdin 1989; Galat et al. 1990).

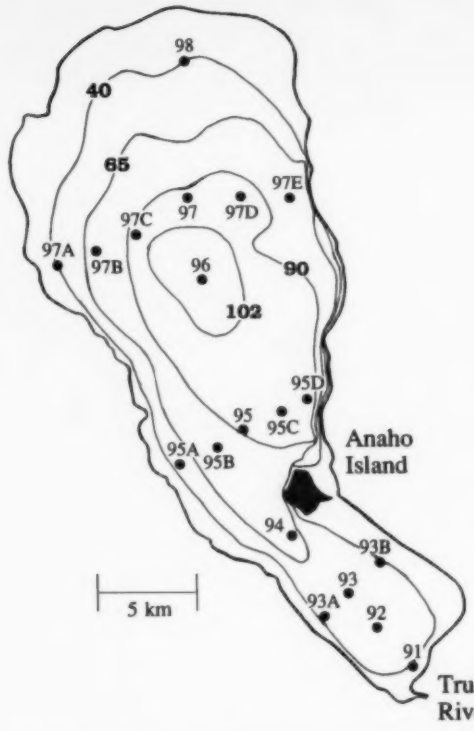

FIG. 1. Station location for 21–23 May 1991 synoptic sampling. Approximate bathymetry (m) of Pyramid Lake estimated from Harris (1970).

Methods

In May 1991, a synoptic survey of 19 stations was conducted along three east–west and one north–south transects (Fig. 1). At each station, profiles for conductivity, temperature, dissolved oxygen, and pH were measured with a Hydrolab Surveyor II system calibrated daily. Discrete water samples for nutrient and particulate matter analyses were obtained from two to six depths (5, 15, 30, 45, 70, and 5 m off the bottom) at each station depending on total depth. A single sample for chlorophyll analysis was taken at 5 m. Additional Hydrolab profiles along the north–south transect (stations 91–98) were obtained during May–October of 1990 and 1991.

Water samples were also collected monthly from October 1989 to December 1991 as part of an ongoing monitoring program at an index station in each basin: station 96 in the center of the main basin at the location of maximum depth (102 m) and station 92 in the southern basin at a depth of 50 m (Fig. 1). At both stations, Hydrolab profiles of conductivity, temperature, dissolved oxygen, and pH were measured. Discrete water samples wre collected from six and 11 depths at stations 92 and 96, respectively, for analysis of nutrient and particulate matter concentrations. Water for chlorophyll analysis was obtained from six (station 92) or nine (station 96) depths in surface waters

(0–25 m). More samples were collected at station 96 because of its greater depth and in conjunction with phytoplankton productivity measurements (data not included).

Dissolved oxygen concentrations were converted to percentage of saturation relative to the atmosphere at the altitude of the lake using the salinity and temperature corrections of Sherwood et al. (1991). Salinity was calculated from conductivity using the fifth-order polynomial established for seawater. Because the ionic composition of Pyramid Lake differs from that of seawater (Galat et al. 1981), salinity values are only approximate, but relative differences over the study period are valid. Values for total dissolved solids (TDS) were calculated from conductivity according to a linear relationship established for Pyramid Lake (Lockheed Ocean Science Laboratory, unpublished data). Sigma-*t* (σ_t, milligrams per litre) was calculated from water density (grams per litre) derived from temperature and salinity by subtracting 1000.

Samples for nutrient analyses were stored frozen until analysis within a few months. Nutrient concentrations were determined spectrophotometrically using standard colorimetric methods. N fractions were determined as follows: ammonium by modified indophenol reaction (Solorzano 1969; Liddicoat et al. 1975), limit of detection (LOD) of 2 $\mu g\ N \cdot L^{-1}$; nitrate + nitrite by cadmium reduction using a shaking technique (Jones 1984), LOD of 1 $\mu g\ N \cdot L^{-1}$; total Kjeldahl N (TKN) as ammonium after acid digestion of raw sample (APHA 1985), LOD of 15 $\mu g\ N \cdot L^{-1}$; and total N (TN) as the sum of TKN and nitrate + nitrite. P fractions were measured as soluble reactive P (SRP, Strickland and Parsons 1972). Total P (TP) was measured in a raw water sample following acid-persulfate digestion (APHA 1985). Phosphate was measured using a raw water sample (total reactive P (TRP)) and 0.45-μm filtrate (dissolved reactive P (DRP)). LOD for P analyses was 1–2 $\mu g\ P \cdot L^{-1}$. DRP, TRP, and TP analyses were modified to remove arsenic interferences (Johnson 1971). Dissolved silicate was measured as reactive silicate (APHA 1985) in 0.45-μm filtrate (LOD of 50 $\mu g\ Si \cdot L^{-1}$).

Particulate matter was collected on Whatman GF/F filters (nominal 0.7 μm) for chlorophyll (Chla) and seston C, N, and P. Filters for seston C, N, and P were prebaked at 450°C for 2 h to reduce organic contamination. Chla was measured fluorometrically after homogenization and a 24-h extraction into cold (4°C) 90% acetone (APHA 1985; LOD of 0.2 $\mu g\ Chla \cdot L^{-1}$). Chla concentrations were corrected for phaeopigments. Particulate N (PN) and C (PC) were determined using a Perkin Elmer model 2400 CHN analyzer (LOD of 2 μg of N and 10 μg of C). Particulate P (PP) was determined as SRP after high-temperature combustion (450–500°C) for 2 h followed by hydrolysis with dilute HCl (Solorzano and Sharp 1980, LOD of 0.02 μg of P). The low LOD for PP results from the concentration of particulate matter on a filter and enables us to reliably separate small differences ($<0.2\ \mu g \cdot L^{-1}$) in PP concentration in lake water as valid trends.

Results

Spatial Patterns for May 1991

Nutrient concentrations during May 1991 were generally constant throughout a large portion of the lake with small regions of enrichment or depletion (Fig. 2). Nitrate + nitrite (NO_3-N) concentrations were the most variable, ranging from < 1 to 20 $\mu g \cdot L^{-1}$, with higher concentrations in the north end of the lake and the western margin of the south basin. In contrast, ammonium (NH_4-N) concentrations were uniformly low throughout the lake with values near the limit of detection. TP variability was generally small with higher concentrations (90–94 $\mu g \cdot L^{-1}$) in the north end of the lake (similar to NO_3-N), but lower values (79–84 $\mu g \cdot L^{-1}$) occurred in the south basin with the minimum value near the mouth of the Truckee River. In contrast with TP depletion near the mouth of the Truckee, SiO_2-Si concentrations were highest (326 $\mu g \cdot L^{-1}$) in this region. TKN concentrations were relatively uniform throughout the lake at 700–812 $\mu g \cdot L^{-1}$ with slightly lower values (666–695 $\mu g \cdot L^{-1}$) on the western side of the northern basin.

For particulate fractions, Chla concentrations were more variable (0.41–1.56 $\mu g \cdot L^{-1}$) than POM (Fig. 2). Generally, Chla was low (0.41–0.62 $\mu g \cdot L^{-1}$) in the deep, middle region of the lake and was higher in the north and south ends. The highest Chla concentration of 1.56 $\mu g \cdot L^{-1}$ occurred in the south end of the lake near Anaho Island. Distributions of PC and PN were similar to Chla with higher concentrations in the north and south ends relative to the middle of the lake. However, variability in PC and PN was much lower than in Chla; the coefficients of variation for PC and PN were 9–11% compared with 39% for Chla. The distribution of PP, although similar in some respects to PC and PN, was highest (8.6–9.8 $\mu g \cdot L^{-1}$) on the western shore of the lake near Anaho Island where PC and PN were low. Since the LOD for PP is $<0.2\ \mu g \cdot L^{-1}$, the observed differences of 2–4 $\mu g \cdot L^{-1}$ are meaningful and indicate that there is a source of P-rich particulate matter along the western shore.

Comparisons for May 1991

Nutrient, Chla, and POM distributions for May 1991 showed that concentrations of some constituents may be elevated or depleted in different regions of the lake. To test for regional differences in concentration, data were grouped by basin and shallow (<60 m) versus deep bottom depth (Table 1). A depth of 60 m was chosen to include stations along the margin of the main basin (95A, 97A, and 98) with shallow stations in the south basin. Two discrete water samples collected at 5 and 15 m depths in surface waters were used in the comparisons. Significant differences were found for only four constituents: NO_3-N, TP, SiO_2-Si, and Chla (Table 1).

NO_3-N concentrations were higher ($P = 0.01$) for shallow stations (<60 m) than for deeper stations (Table 1). The lack of a significant difference between basins for NO_3-N concentration despite mean values similar to those found for the depth comparison was due to greater variability in the former comparison. It also indicates that the source of variability was operating in shallow regions throughout the lake rather than only in the smaller, southern basin. This result is not surprising because elevated NO_3-N concentrations were measured in both the north and south ends of the lake (Fig. 2).

The main spatial variability for TP and SiO_2-Si appeared to be along a north–south gradient of the lake. Although both comparisons for each constituent revealed significant differences (Table 1), the comparisons between basins yielded greater differences and hence higher *t* values for Student's *t*-test; *t* values were higher for TP (4.51 versus 2.47) and SiO_2-Si (6.28 versus 2.75) in the basin comparison compared with values grouped by station depth.

Elevated Chla concentrations in the south basin and in shal-

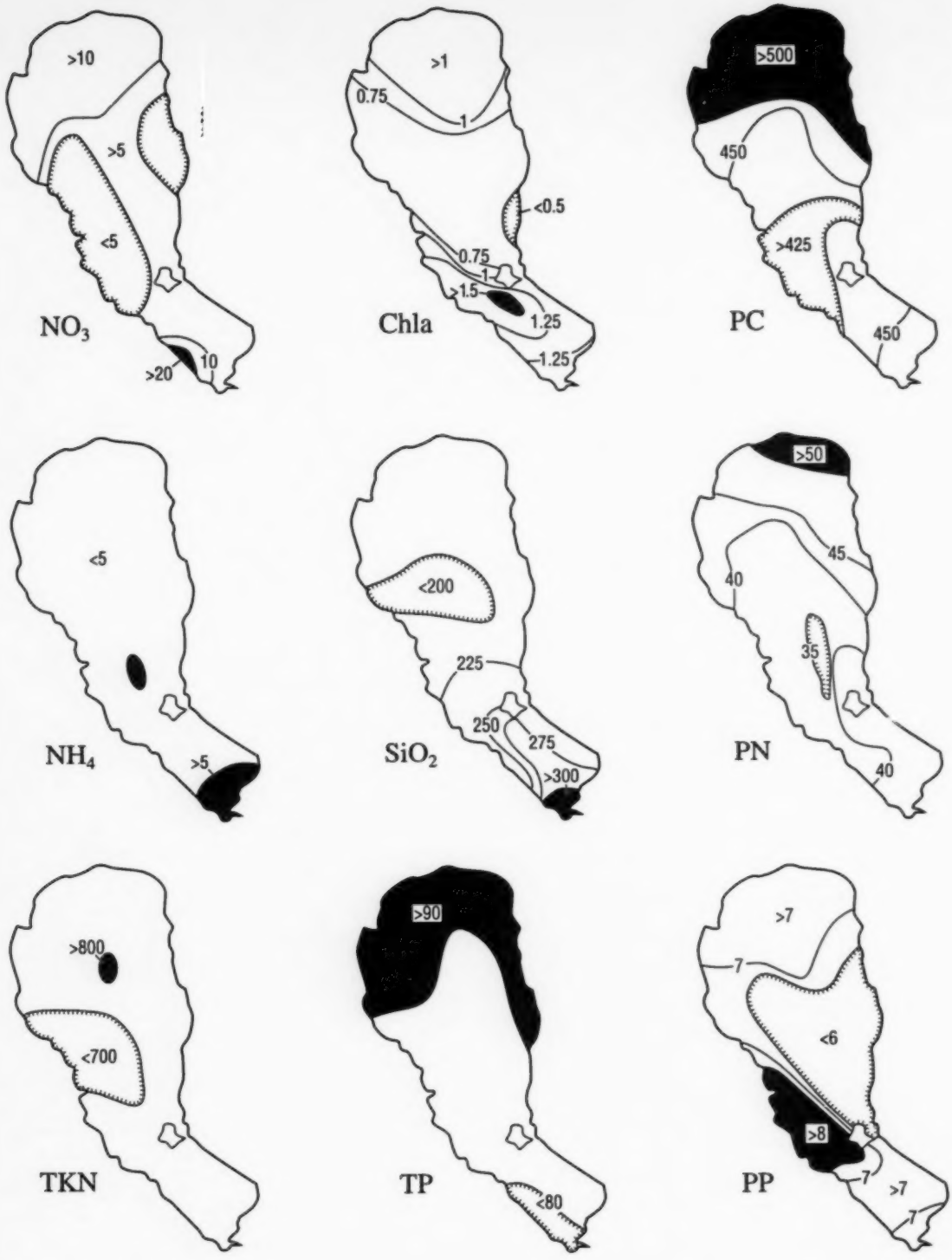

FIG. 2. Distributions of nutrients (NO_3-N, NH_4-N, TKN, SiO_2-Si, and TP), Chla, and particulate matter (PC, PN, and PP) in surface waters during 21–23 May 1991. Contours are in $\mu g \cdot L^{-1}$ and are based on data collected at 19 stations (see Fig. 1).

TABLE 1. Comparison of mean (± SD) nutrient, Chla, and POM concentrations ($\mu g \cdot L^{-1}$) for May 1991 between the north and south basins and between shallow (<60 m) and deep water stations in Pyramid Lake. In the basin comparison, the basins are separated at Anaho Island with station 94 included in the south basin. Mean values were compared with Student's *t*-test. Significantly higher values are indicated with asterisks: $*P < 0.05$; $**P < 0.01$.

	Basin comparison		Depth comparison	
Constituent	South (n = 11)	North (n = 25)	<60 m (n = 15)	>60 m (n = 21)
NO_3-N	9.6 ± 7.3	6.3 ± 3.7	10.0 ± 6.7*	5.4 ± 2.7
NH_4-N	4.3 ± 1.4	3.4 ± 1.8	4.0 ± 1.3	3.5 ± 2.0
TKN	753 ± 87	727 ± 60	735 ± 77	736 ± 65
TP	81.8 ± 3.4	88.2 ± 4.7**	83.6 ± 5.0	87.8 ± 4.7*
SiO_2-Si	278 ± 34**	210 ± 20	254 ± 45**	217 ± 29
Chla	1.29 ± 0.16**	0.76 ± 0.29	1.22 ± 0.09**	0.76 ± 0.36
PC	451 ± 28	451 ± 42	457 ± 34	443 ± 35
PN	40.6 ± 2.9	39.9 ± 4.9	41.1 ± 3.1	39.2 ± 4.9
PP	7.0 ± 0.9	6.3 ± 1.3	6.8 ± 1.0	6.4 ± 1.3

low stations were highly significant (Table 1), although, as with TP and SiO_2-Si, the basin comparison was more significant than the depth comparison. However, the difference in t values for the Chla comparisons was less pronounced (4.69 versus 3.85) than for TP and SiO_2-Si listed above. This suggests that the low number of shallow stations sampled in the northern basin gives insufficient power to separate whether the observed differences in Chla are due to water depth or a basin effect.

Evidence for Upwelling

Elevated NO_3-N concentrations along the northern and southwestern margins of Pyramid Lake in May 1991 (Fig. 2) indicate the potential for upwelling of nutrient-rich hypolimnetic waters in these regions. NO_3-N is a good tracer of processes like upwelling in Pyramid Lake, since surface concentrations during summer stratification are generally $<20\ \mu g \cdot L^{-1}$ whereas bottom waters increase to $>100\ \mu g \cdot L^{-1}$ (Galat et al. 1981; Hamilton-Galat and Galat 1983; Lebo et al. 1992). Wind records from Sutcliffe, Nevada, on the western shore of the lake support the potential for upwelling along these margins prior to our synoptic sampling; for 4 d before our field study, winds were continuous at 5–9 $m \cdot s^{-1}$ out of the west (Pyramid Lake Fisheries, unpublished data). This is a favorable wind direction and of sufficient duration to initiate upwelling along the northern and southwestern margins of the lake (Csanady 1968; Simons and Schertzer 1987).

Surface water temperatures during May 1991 (Fig. 3) support upwelling prior to our synoptic sampling. Along the northern and southwestern margins of the lake, water temperatures were lower (<10°C) than in the main body of the lake (10–11°C). Although these spatial changes in water temperature are small, they are consistent with upwelling of colder waters from below the shallow (<20 m), weak (<5°C) thermocline found during our field study.

The origin of water upwelled along the northern margin of the lake, at least in May 1991, appears to be from intermediate depth rather than deep waters. Along the north–south axis (stations 91–98) of the lake (Fig. 4), the 9–10°C isotherms clearly bend toward the surface in the northern reaches (30–40 km) as do the 8 and 10 $\mu g \cdot L^{-1}$ isopleths of NO_3-N concentration. The source of upwelled water indicated by both constituents was intermediate depth (20–40 m) water in the center of the lake (20–30 km). Although the upwelling of bottom waters near the northern margin could produce the temperature distribution we observed, NO_3-N concentration would not be elevated due to low NO_3-N concentration ($<5\ \mu g \cdot L^{-1}$) below 30 m.

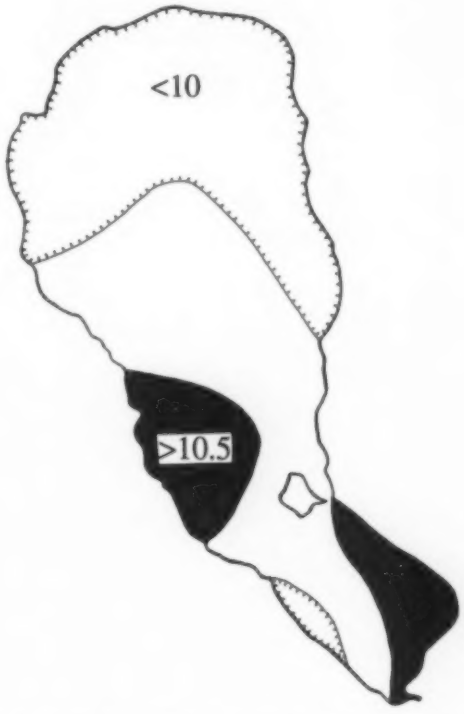

FIG. 3. Isotherms for surface waters (5 m) during 21–23 May 1991. Contours are in °C and are based on data collected at 19 stations (see Fig. 1).

Seasonal Differences in Basins

Monthly data collected at index stations 92 and 96 during October 1989 to December 1991 were used to determine if spatial trends identified for May 1991 were typical for the lake or part of transient seasonal variations. To simplify subsequent

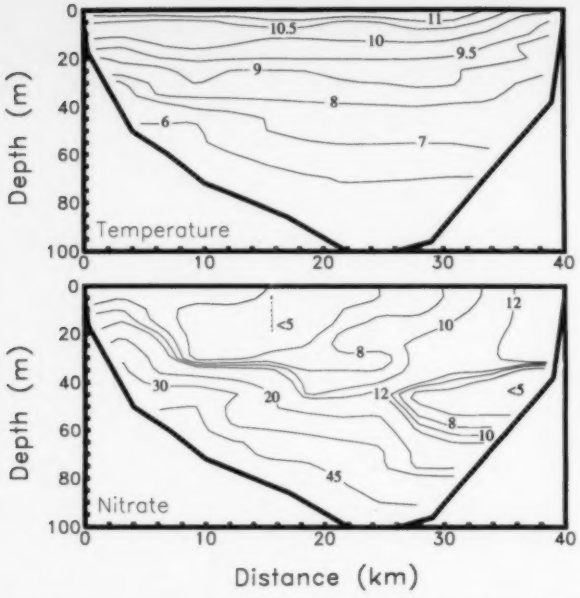

FIG. 4. Water temperature (°C) and nitrate concentration ($\mu g \cdot L^{-1}$) for 21–23 May 1991 contoured versus depth and distance from Truckee River along the north–south axis of the lake. Data from stations 91–98 were used to prepare the plots. The north end of the lake is on the right side of the panels.

discussions, we will simply refer to the two stations as shallow (92) or deep (96). For each location, mean volume-weighted surface concentrations of constituents were calculated from depth profiles of three or four discrete points for nutrients and POM or six to nine points for Chla. The difference in mean surface concentration between stations was computed for each sampling date, and then this difference was averaged over the entire sampling period. A pattern was inferred if mean difference between stations for all sampling dates was significantly different from zero. In these comparisons, the difference in concentration between stations was used rather than mean concentrations due to large seasonal variations in several of the constituents. For example, mean NO_3-N concentrations for the deep station varied from 3 to 55 $\mu g \cdot L^{-1}$ between January and April 1991. Lebo et al. (1992) described seasonal variations in nutrients, POM, and Chla for 1990.

The mean differences between concentrations at the shallow and deep stations were not significantly different from zero for all constituents except SiO_2-Si. The only spatial trend observed in May 1991 that was consistently true for the entire study period was the enrichment of SiO_2-Si in the south basin relative to the main body of the lake ($P = 0.03$). For all constituents except SiO_2-Si and NH_4-N, mean differences between stations were small (<5%) relative to the midpoint of seasonal variations. The apparent large relative difference in NH_4-N concentration between basins was due to low concentrations, which were generally <5 $\mu g \cdot L^{-1}$ throughout most of the year.

The differences in nutrients, POM, and Chla concentrations between the shallow and deep stations (deep minus shallow) were plotted against time of year to ascertain seasonal variations. For all constituents except Chla, NO_3-N, and TP, no obvious seasonal patterns were apparent, including SiO_2-Si.

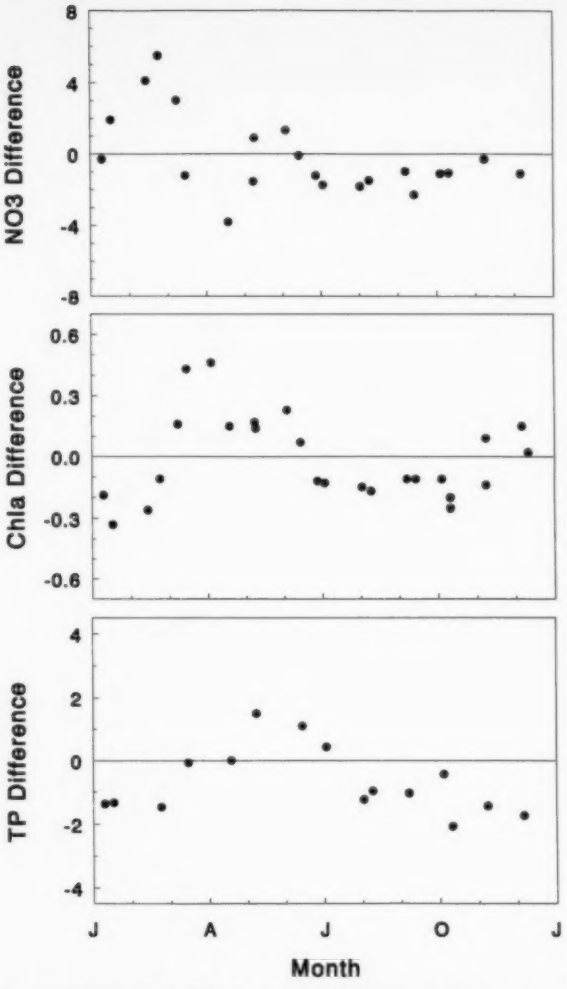

FIG. 5. Difference in NO_3-N, Chla, and TP concentrations between deep and shallow index stations (deep minus shallow) plotted by time of year. Values are three-point moving averages and are in $\mu g \cdot L^{-1}$.

The seasonal plots for deep minus shallow indicate that spatial differences for Chla, NO_3-N, and TP during May 1991 were part of general seasonal patterns (Fig. 5). For NO_3-N, higher concentrations at the shallow station relative to the main body of the lake were common during most of the year, except during winter mixing during January–March. A similar pattern was observed for Chla, although the timing of transitions was different. For Chla, higher concentrations at the deep station (March–May) followed winter mixing of NO_3-N to the surface. The seasonal pattern for TP was similar to NO_3-N and Chla with higher concentrations at the shallow station during most of the year, but the timing of transitions appeared to be unrelated to winter mixing. Whereas the transition periods for NO_3-N (March) and Chla (May) occurred during the spring, the transition from higher TP concentrations at the deep station to lower values relative to the shallow station occurred during summer (July).

Differences in light and nutrient availability between basins may explain the observed seasonal pattern for Chla. Because

the south basin is small relative to the main body of the lake, strong winter–spring winds (Galat et al. 1981; Pyramid Lake Fisheries, unpublished data) should have a greater impact on water clarity than in the middle of the lake. Indeed, light extinction was higher at the shallow station relative to the deep one between January and April (Fig. 6), although mean difference was only significant ($\alpha = 0.05$) for January–February due to large variations in light extinction at the shallow station during March and April of different years (coefficient of variation = 19%). The result of higher light extinction at the shallow station during January–April was that light was less available for phytoplankton in the south basin compared with in the main body of the lake. For March–April, the average depth of 1% below-surface irradiance was 17.7 m at the deep station compared with only 15.3 m for the shallow station. In wet years, water clarity in the south end of the lake may be further reduced due to increased turbidity associated with high river inflow (Galat 1986).

Another difference between the basins in the spring is that phytoplankton have a larger (deeper) reservoir of nutrients to utilize in the center of the lake. During the unstratified to partially stratified period, nutrients from below the photic zone are mixed into surface waters. Therefore, the greater depth at the deep station provides a larger pool of nutrients from which to draw. More nutrients to fuel the spring bloom could potentially contribute to a longer boom in the main body of the lake relative to the south basin. However, the merit of this assertion cannot be tested with our monthly data.

During the summer, the south basin appeared to be more productive with higher nutrient and Chla concentrations. The smaller size of the south basin may contribute to this apparent difference. Since upwelling was shown to occur along the margins of both basins, this source of nutrients would be available to surface waters throughout the lake. In the south basin, the relative impact of processes such as upwelling and eddy diffusion would be greater than in the main body of the lake due to its smaller size and volume. It should be noted that the relatively small spatial differences observed during 1989–91 would, of course, be overwhelmed by heterogeneity associated with *N. spumigena* during large blooms.

Discussion

Importance of Spatial Variability

Knowledge of spatial variability of nutrients, POM, and Chla concentrations is important to our understanding of lake processes. Generally, monitoring of water quality in lakes consists of sampling a few fixed locations at different times of year. The detection and verification of trends determined from these sparse data sets is constrained, to some extent, by a knowledge of the variability or "patchiness" of the constituents of interest. Alternatively, an understanding of the spatial variability of nutrients and POM facilitates developing an appropriate sampling strategy.

Spatial patterns of nutrients, POM, and Chla concentrations can indicate important sources of different constituents. For example, the link between phytoplankton production and nutrient concentrations can be used to infer higher nutrient supply in areas of elevated Chla. This same concept can be further exploited by examining spatial changes in the elemental ratio of POM. Generally, seston elemental composition is used to infer nutrient deficiency of plankton (Healey and Hendzel 1980; Lean et al. 1989; Dodds and Priscu 1990; Kilham 1990),

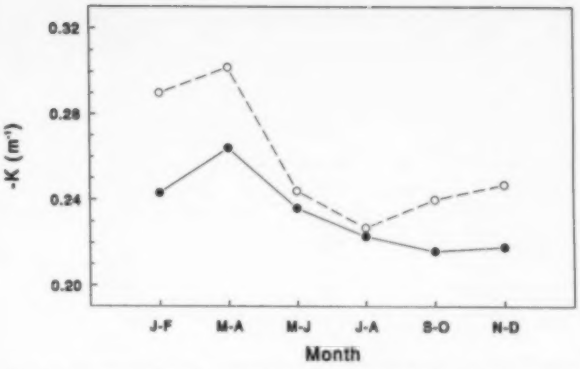

FIG. 6. Average extinction coefficient for light at shallow (open circles) and deep (solid circles) index stations. Values are bimonthly averages (e.g., January–February) based on three to nine observations during the current drought (1987–91).

but spatial variations in seston composition can also identify nutrient sources. Further, we can use information about nutrient sources to identify processes that are important in nutrient supply rate to the photic zone.

The applicability of spatial trends identified in this study is potentially constrained by drought conditions afflicting the region over the past 5 yr. During 1989–91, Truckee River inflow, the only major source of freshwater to the lake, was only 5% of mean flow during 1972–87 (Galat 1990). Low inflow during the present study is contrasted by an extremely wet period during 1982–86. By comparing spatial patterns described for 1989–91 with past data collected during the wet period in the mid-1980's, we can ascertain the limitations of our observations. Chemistry and Hydrolab profiles of Pyramid Lake water were measured on several occasions in 1983–84 during high flow conditions (D.L. Galat et al., unpublished data). The spatial patterns for SiO_2-Si and TP during the wet period were similar to the distributions described in this study, although north–south gradients were greatly magnified. For example, SiO_2-Si concentration in June 1984 was 4.9 $mg \cdot L^{-1}$ near the mouth of the Truckee River compared with 0.5 $mg \cdot L^{-1}$ in the middle of the lake. Therefore, it appears that low inflow during the present study did not affect the spatial patterns of constituents such as SiO_2-Si and TP, only the magnitude of north + south gradients.

A second major source of "patchiness" in Pyramid Lake, *N. spumigena* blooms during summer–fall (Galat and Verdin 1988, 1989; Galat et al. 1990), was also minimal during our study period. However, *N. spumigena* blooms are generally of short duration (2–4 wk, Galat and Verdin 1989; Galat et al. 1990). Although spatial distributions of Chla and POM are particularly affected by *N. spumigena* blooms (see Galat et al. 1990 for Chla distributions for 1972–87 blooms), phytoplankton production probably depends on internal nutrient loading during most of the year (Hamilton-Galat and Galat 1983; Lebo et al. 1992). We can take advantage of small *N spumigena* blooms during 1989–91 to examine these internal processes that might otherwise be masked.

Wind-Driven Circulation

Given the meteorological conditions of our sampling period, wind-driven processes contributed greatly to spatial variability around the lake. Prevailing westerly winds across the main axis

of Pyramid Lake generate the potential for upwelling of subsurface waters to the left of wind direction, or in the north end of the lake, by pushing surface waters to the right in the Northern Hemisphere (Csanady 1968; Simons and Schertzer 1987). Temperature (Fig. 3) and nitrate (NO_3-N, Fig. 2) distributions suggest that upwelling was occurring during May 1991 after 4 d of strong northwesterly winds (>5 $m \cdot s^{-1}$). Simons and Schertzer (1987) described a simple spatial pattern for Lake Ontario of upwelling on the left margin of the basin and downwelling on the right side. However, this pattern is too simplistic for Pyramid Lake due to its irregular shape. Data from May 1991 indicate that upwelling also occurred along the southwestern margin of the lake in the south basin (Fig. 2 and 3).

With records from Sutcliffe on the western shore of Pyramid Lake (Pyramid Lake Fisheries, unpublished data) were used to assess the frequency of potential upwelling events. Clearly, our synoptic sampling in May 1991 indicated that upwelling occurred after 4 d of strong winds. On a shorter time scale, Strub and Powell (1987) attributed upwelling at Lake Tahoe, another large lake in the western United States, to diurnal summer wind fields. Given that the surface areas of Pyramid Lake and Lake Tahoe are nearly identical, diurnal wind fields may also apply at Pyramid. For the purposes of this study, we defined conditions favorable for upwelling as periods with sustained winds >5 $m \cdot s^{-1}$ for 3 d or more, which is a conservative estimate of upwelling potential. The evaluation of potential upwelling was limited to the period when upwelling would increase surface NO_3-N concentration; data were only used from March–November when surface NO_3-N was reduced and bottom concentrations were high (Galat et al. 1981; Hamilton-Galat and Galat 1983; Lebo et al. 1992). Wind data were available at Sutcliffe for 1986–91.

Meteorological conditions favorable for upwelling in Pyramid Lake occurred frequently during 1986–91. For the entire period, 31 occasions favorable for upwelling were found with 65% of total events occurring during March–May. Wind events occurred most frequently during May with 1.8 events on average each year. During May 1987 and 1991, conditions favorable for upwelling occurred frequently; winds favorable for upwelling occurred on three and four occasions during May 1987 and 1991, respectively. Overall, there was a large difference between years with potential events ranging from one in 1990 to eight in 1987.

Water temperature profiles from the north end of the lake also support additional upwelling episodes during 1989–91. Using Hydrolab data collected along the north–south transect line of the lake, particularly stations 96–98 (Fig. 1), evidence for additional upwelling events was assessed. For each transect, upwelling was inferred if the depth where water temperature was <7°C was shallower at station 98 than at stations 96 and 97. A water temperature of <7°C was used, since it only occurred below the thermocline after the onset of stratification around April (Lebo et al. 1992). Probable upwelling was suggested for three occasions out of nine total transects examined for June–September 1990 and May–August 1991. In addition to the event in May 1991, upwelling was inferred from profiles obtained in June and September 1990. For these two occasions, water temperature at a depth of 40–45 m at station 96 occurred at a depth of 30–35 m at station 98. No change or an increase in temperature at comparable depths between stations 96–98 occurred for the remaining six dates for which Hydrolab data were available.

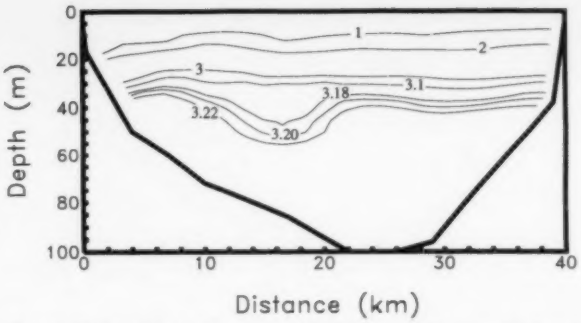

FIG. 7. Sigma-t ($mg \cdot L^{-1}$) contoured for August 1990 versus depth and distance from Truckee River along the north–south axis of the lake. Data from stations 91–98 were used to prepare the plot.

The two probable upwelling events for 1990 indicated by temperature profiles contrast with our conclusion of only one potential upwelling event during March–November 1990 based on wind records. This suggests that our criterion of 3 d of sustained winds of >5 $m \cdot s^{-1}$ is too conservative. Further, the wind records used are compiled during the morning whereas winds are generally greatest in the afternoon during summer months. It is quite possible, therefore, that the upwelling of nutrient-rich waters in the north end of the lake occurs frequently during spring–fall.

There are two main consequences of periodic upwelling events in Pyramid Lake: surface enrichment of NO_3-N and subsurface return flow from south to north. Because N is the nutrient most limiting to phytoplankton growth in Pyramid lake (Hutchinson 1937; Galat et al. 1981; Hamilton-Galat and Galat 1983; Reuter et al. 1993), upwelling of NO_3-N-rich waters to the surface may serve to reduce the impact of low surface NO_3-N concentrations during summer. This source of NO_3-N to surface waters during spring–fall may even forestall intense N limitation in some years; however, definite conclusions regarding the overall importance of upwelling to N cycling require further study.

Subsurface northward flow in Pyramid Lake confuses the interpretation of vertical profiles by introducing a lateral transport component. Water density (σ_t) contoured along the north–south axis of the lake for August 1990 indicates water movement toward the middle body of the lake; isopycnal lines for 3.18–3.22 $mg \cdot L^{-1}$ in the south basin (3–10 km) are sloped downward toward the middle of the lake (Fig. 7). The impact of this lateral subsurface transport is the injection of nutrient-poor shallow waters from the south basin into the hypolimnion of the north basin. For example, TRP concentration at the deep station was lowest (47–51 $\mu g \cdot L^{-1}$) at the surface and generally increased with depth, as is typical for the stratified period (Hamilton-Galat and Galat 1983; Lebo et al. 1992). However, there was a relative minimum of 55 $\mu g \cdot L^{-1}$ in the hypolimnion at 75 m compared with concentrations >60 $\mu g \cdot L^{-1}$ above and below this level. The most likely explanation for this hypolimnetic relative minimum in TRP is lateral transport of water from the south basin.

Dust Transport

The wind is also an important pathway for the input of vegetation and dust to saline lakes. Galat et al. (1981) reported

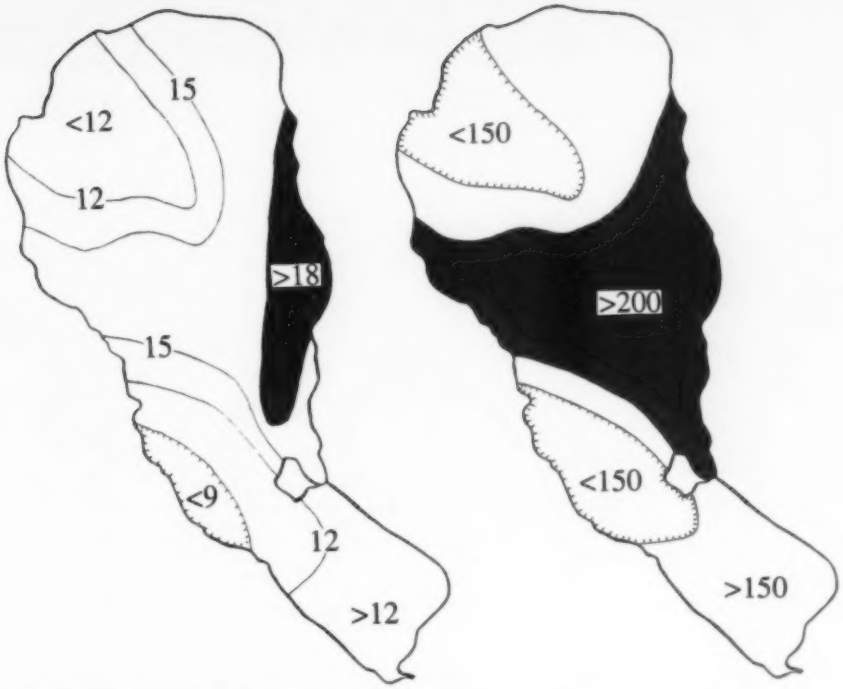

FIG. 8. Distributions of (left) N:P and (right) C:P elemental ratios of seston during 21–23 May 1991. Contours are based on data collected at 19 stations (see Fig. 1).

intense dust storms at Pyramid Lake, especially in the spring, with prevailing westerly winds in excess of 20 $m \cdot s^{-1}$. These winds transport tumbleweeds, soil, and other small particles into the lake, with tumbleweeds accounting for up to 6% of total annual C inputs (Galat 1986). Although dust input to the lake is high, Galat (1986) reported that it was insignificant in the C budget because it contained only 3 $\mu g\ C \cdot g^{-1}$. However, the input of carbonate-rich, alkaline dust to the lake may be an important source of P.

Distributions of POM during May 1991 (Fig. 2) suggest that there was a source of P-rich and N- and C-poor particulate material along the western margin of the lake. Contour plots of the elemental ratios of POM indicate that POM is P rich in the northwest corner of the lake and to the west of Anaho Island (Fig. 8); C:P and N:P ratios of POM were <150 and <12, respectively, compared with higher values throughout the main body of the lake. These are the leeward margins of the lake during westerly winds. Because wind transport of material over lakes usually decreases exponentially with distance from shore (Cole et al. 1990), the spatial patterns for C:P and N:P ratios are consistent with wind transport of dust.

A sediment trap was deployed at the deep station during April–May 1991 to collect material leaving surface waters (30 m). The elemental composition of this material was used to estimate the contribution of inorganic PP to total P flux. Assuming that the C:P ratio of plankton in Pyramid Lake was relatively constant throughout the year (the lake is C and P rich), the contribution of dust was estimated by subtracting the organic fraction from total P flux. Since the C content of dust is low (3 $\mu g\ C \cdot g^{-1}$, Galat 1986), we assumed that all C in sediment trap material was from plankton and calculated a P equivalent using the average C:P ratio (245 ± 45; $n = 7$) for surface waters during summer–fall of 1990–91. The remaining P in sinking material was assumed to be inorganic.

Particulate material sinking out of surface waters during spring 1991 was P rich, suggesting that a large fraction of particle-bound P was inorganic. For April–May 1991, the C and P contents of trap material were 55.1 and 1.29 $mg \cdot g^{-1}$, respectively, yielding an inorganic contribution of 0.71 mg $P \cdot g^{-1}$, or 55% of the total. This indicates that the total flux of inorganic P from surface waters was 1.3 mg $P \cdot m^{-2} \cdot d^{-1}$, since total sedimentation was 1.8 $g \cdot m^{-2} \cdot d^{-1}$. A dry deposition collector has been deployed at the deep station to provide a direct comparison for sediment trap data in the future.

The apparent eolian input of P calculated above from sediment trap data for April–May is similar to fluvial P inputs to the lake. Galat (1990) reported that the average annual loading of P to Pyramid Lake during 1982–87 was 396 mg $P \cdot m^{-2} \cdot yr^{-1}$. Extrapolating our estimate above to an annual rate yields approximately 470 mg $P \cdot m^{-2} \cdot yr^{-1}$, or 120% of fluvial inputs for 1982–87. It should be noted that we expect the input of dust to be greatest in the spring when winds are generally stronger (Galat et al. 1981; Pyramid Lake Fisheries, unpublished data), and therefore, the extrapolation of our single estimate to an annual rate is not valid. The redistribution of fine bottom sediments to deeper regions of the lake could also bias our result. We use this estimate only to emphasize that P associated with eolian dust may be a neglected dominant source of P to the lake. It is interesting to note that our particulate matter flux for April–May 1991 (1.5 g inorganic $\cdot m^{-2} \cdot d^{-1}$) is much smaller than the rate of dry deposition for January–March 1977 (31 $g \cdot m^{-2} \cdot d^{-1}$) derived from Galat (1982). This suggests that input of dust can be quite large during certain years.

Summary

Despite low productivity and drought conditions, our observations appear to describe fundamental processes of Pyramid Lake. During 1989–91, spatial heterogeneities in nutrients and POM were associated with river inflow and wind-driven processes such as upwelling and dust transport. These processes should be important during all years, since periodic strong winds are characteristic for the region and river inflow is generally much higher. Further, the importance of wind-driven processes extends beyond our present observations and may include regulation of *N. spumigena* production (Galat and Verdin 1988, 1989). Clearly, an understanding of phytoplankton ecology in Pyramid Lake requires the inclusion of physical processes associated with wind forcing.

Acknowledgements

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Effects of Water Velocity, Group Size, and Prey Availability on the Stream-Drift Capture Efficiency of Blacknose Dace, *Rhinicthys atratulus*[1]

Jeffrey A. Tyler[2]

Ecology and Animal Behavior Group, Department of Biological Sciences, State University of New York at Albany, Albany, NY 12222, USA

Tyler, J.A. 1993. Effects of water velocity, group size, and prey availability on the stream-drift capture efficiency of blacknose dace, *Rhinicthys atratulus*. Can. J. Fish. Aquat. Sci. 50: 1055–1061.

In laboratory flow-tank experiments, I determined the effects of water velocity, group size, and prey arrival rate on the proportion of drifting prey a group of blacknose dace, *Rhinicthys atratulus*, captured. Water velocity, group size, and the interaction between the two accounted for significant proportions of the variance found in the stream-drift capture efficiency of the fish. Neither prey arrival rate nor any of the interactions which included prey arrival rate explained a significant proportion of the variance in the capture data. I present a regression relating water velocity and group size to the proportion of drift items fish capture that should be valuable for future studies of fish habitat selection. Further analysis of the regression found an optimal water velocity for drift-feeding blacknose dace at between 24 and 27 $cm \cdot s^{-1}$. Because of the significant interaction between water velocity and group size, the advantage of foraging in sites with optimal water velocity decreased as the number of intraspecific competitors increased.

Grâce à des expériences de laboratoire faisant appel à des réservoirs avec circulation d'eau, j'ai déterminé les effets de la vitesse de l'eau, de la taille du groupe et du taux d'arrivée des proies sur la proportion de proies dérivantes capturées par un groupe de naseux noirs, *Rhinicthys atratulus*. La vitesse de l'eau, la taille du groupe et les interactions entre les deux expliquent une part importante de la variance observée dans l'efficacité de la capture dans le courant des poissons. Ni le taux d'arrivée des proies ni aucune interaction faisant intervenir ce facteur n'ont expliqué une part importante de la variance observée dans les données de capture. Je présente une analyse de régression qui met en relation la vitesse de l'eau et la taille du groupe avec la proportion d'organismes dérivants capturés par les poissons et qui pourrait être utile dans les études futures sur la sélection des habitats de poisson. Une analyse plus poussée de la régression a révélé une vitesse optimale de l'eau pour l'alimentation du naseux noir aux dépens des organismes dérivants, vitesse qui se situe entre 24 et 27 $cm \cdot s^{-1}$. En raison de l'interaction importante entre la vitesse de l'eau et la taille du groupe, l'avantage de s'alimenter dans des endroits où la vitesse de l'eau est optimale diminue au fur et à mesure que le nombre de naseux augmente (compétition intraspécifique).

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Factors affecting the foraging efficiency of stream fishes feeding on drifting food have received little attention from researchers (see reviews by Dill 1983, 1987) even though studies have shown that fish compete for and defend stream positions based partly on their value as food acquisition sites (Jenkins 1969; Fausch and White 1981; Bachman 1984; Fausch 1984; Grant and Noakes 1987, 1988; Grant et al. 1989). A few studies have addressed the relationship between stream habitat characteristics and fish foraging efficiency and its importance in fish microhabitat selection (Wilzbach et al. 1986; Hill 1989; Hughes and Dill 1990). Water velocity has received particular attention because fishes in sites with fast water velocities have high prey encounter rates (Chapman and Bjornn 1969; Symons 1976; Li and Brocksen 1977; Wańkowski and Thorpe 1979; Fausch and White 1981; Fausch 1984). However, Grant and Noakes (1987, 1988) showed that foraging attempts by brook trout (*Salvelinus fontinalis*) vary with water velocity according to a concave relationship. Trout increased foraging attempts as water velocity increased up to 7.5 $cm \cdot s^{-1}$ and decreased foraging attempts as water velocity increased above 7.5 $cm \cdot s^{-1}$. Also, Hill (1989) demonstrated that solitary rainbow trout (*Oncorhynchus mykiss*) and small groups of rosyside dace (*Clinostomus funduloides*) have low foraging efficiencies at fast water velocities and that this low foraging efficiency affects the fishes' microhabitat selection. Hughes and Dill (1990) modeled foraging ability in Arctic grayling (*Thymallus arcticus*) as a function of reactive distance, water velocity, and water depth. Their model predicted a decrease in foraging efficiency as water velocity increased, and more accurately predicted grayling microhabitat selection than did alternative models that did not vary foraging ability with water velocity.

Wilzbach et al. (1986) examined the effect of surface light and substrate crevices in logged and unlogged areas of forest to determine their effect on cutthroat trout (*Oncorhynchus clarki*) foraging success. Their work differs from most studies

[1]Contribution No. 4109 of the Environmental Sciences Division, Oak Ridge National Laboratory.

[2]Current address: Environmental Sciences Division, Oak Ridge National Laboratory, Box 2008, Building 1505, MS 6038, Oak Ridge, TN 37831-6038, USA.

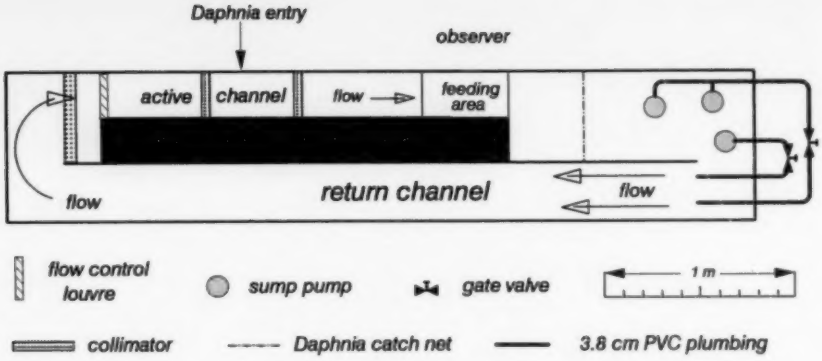

FIG. 1. Overhead view of laboratory flow tank. See text for details.

relating habitat characteristics to fish foraging because it explores the effect of each characteristic and the effect of interactions between characteristics on foraging success. Wilzbach et al. (1986) found that increased surface light and a decreased number of substrate crevices increased trout foraging efficiency, but that the overall changes in the habitat from logging altered these effects. Although Wilzbach et al.'s (1986) data do not allow them to test the strength of the interactions between logging, surface light, and substrate crevices, the interaction of habitat characteristics appears to have an important effect on trout foraging efficiency. In fact, it would be surprising to find that the effects of habitat characteristics on foraging efficiency in general do not show significant interactions.

In this study, I examine the effect of water velocity, food availability, and the number of intraspecific competitors on the foraging efficiency of blacknose dace, *Rhinicthys atratulus*, feeding on drifting *Daphnia magna* in a laboratory flow tank. I designed the experiment to investigate both the main effects of each individual factor and also the interactions between factors on dace foraging efficiency. In the laboratory "habitat," water moved at a uniform velocity throughout, and prey enter according to a stochastic delivery schedule. Blacknose dace that are not in breeding condition do not show dominance or agonistic interactions (Bartnik 1970; Gibbons and Gee 1972; Symons 1976). Therefore, changes in the number of competing fish (hereafter referred to as group size) altered the level of exploitative competition the fish experienced. The laboratory flow tank allowed me to separate changes in food availability from changes in water velocity and, thus, to manipulate each factor independently.

Methods

Laboratory Flow Tank

I conducted all experimental trials in a laboratory flow tank (Fig. 1) equipped with a *Daphnia* delivery wheel (Fig. 2) patterned after that of Beacham (1988). This system allowed me to manipulate water velocity, group size, and the availability of *Daphnia*. The tank measured 13 m × 80 cm × 40 cm consisting of an active channel in which I controlled water velocity and delivered *Daphnia* and a return channel for recirculating the water. Within the active channel the fish were confined to a feeding area 45 × 25 cm with a 10-cm water depth. Throughout the active channel lay a substrate of gravel 2–4 mm in diameter and 10 cm deep. The remainder of the flow tank did not have gravel in it.

I constructed the entire flow tank of Plexiglas 6 mm (0.25 in.) thick. A rigid plastic grid covered with 6-mm hardware cloth formed barriers upstream and downstream of the feeding area. These barriers allowed *Daphnia* to pass through the feeding area while confining the fish. A 0.2-mm net prevented *Daphnia* not captured by the fish from recirculating through the tank and back into the feeding area.

The *Daphnia* delivery wheel (Fig. 2) consisted of 6-mm-thick Plexiglas, 61 cm in diameter. It held 500 glass tubes, 2.5 cm long, 6 mm inside diameter, arranged in five concentric rings of 100 tubes. Prior to an experimental trial, I placed one *Daphnia* and enough water to fill the glass tube into each of 100 tubes. Surface tension held the water and *Daphnia* in the tube until it was delivered. *Daphnia* were delivered by rotating the wheel in front of an air jet which pushed the *Daphnia* and water out of the tube from between 30 and 40 cm above the water surface. After landing in the water, the *Daphnia* drifted 95 cm to the feeding area.

Three sump pumps created the tank's recirculating water flow. Gate valves in the plumbing connected to the pumps along with a flow-control louvre regulated water velocity. For each trial, water velocities in the middle and on each side of the feeding area, measured with a Gurley Pygmy flow meter, lay within 1 $cm \cdot s^{-1}$ of the velocity prescribed for the trial. Collimators made of a rigid plastic grid greatly reduced turbulence and eddies in the water current and allowed *Daphnia* to pass through the active channel without being caught in vortices (Vogel 1981, appendix 1).

An opaque canopy covered the entire apparatus including the area where the observer sat, and only the feeding area was lit with a fluorescent lamp. This lighting system apparently prevented the fish from observing me during a trial, since I never saw the fish react to any of my movements.

Holding and Preparation of Fish for Experiments

I collected all of the fish used in this study from the Lisha Kill, a second-order stream in Niskayuna, NY, which flows into the Mohawk River. In the areas from which I removed fish, water velocities ranged from <5 to 60 $cm \cdot s^{-1}$. In the laboratory the fish stayed in 115-L aquaria at room temperature (21°C) under a 12 h light : 12 h dark photoperiod. While not involved in experiments, the fish received flaked food ad libi-

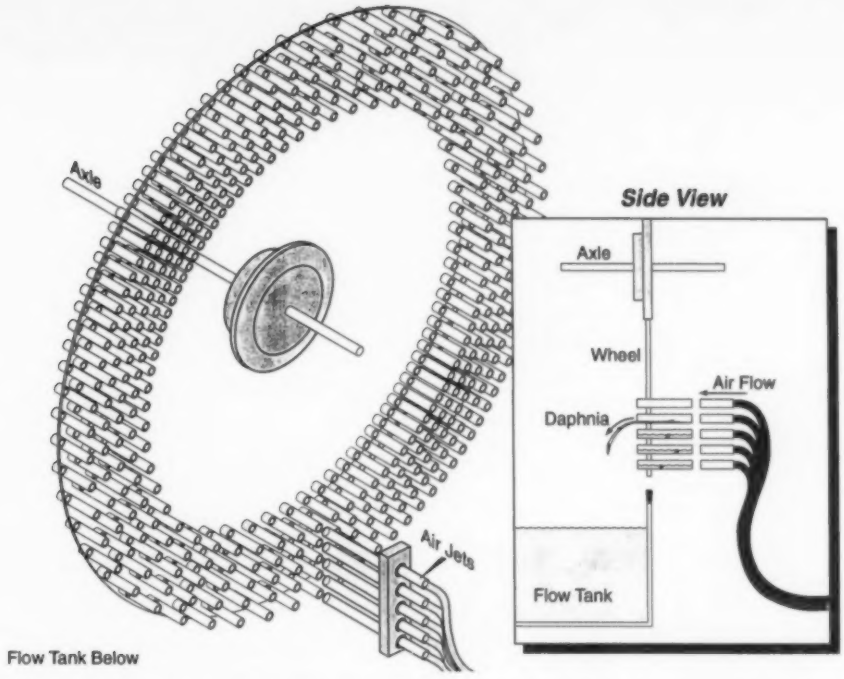

FIG. 2. Diagram of *Daphnia* delivery wheel (not to scale); three-dimensional perspective and side view. Wheel rotates on its axle to move one set of tubes at a time in front of the bank of air jets. Only one air jet is on at a time. See text for further details.

tum. While involved in preparatory or experimental trials, the fish were fed *Daphnia*, and only when they were in the flow tank.

In preparatory trials, groups of 12–15 fish were taken from the holding tanks and placed into the flow tank with the water velocity set between 10 and 40 $cm \cdot s^{-1}$. For these trials, I delivered *Daphnia* with a system similar to Abrahams' (1989) and allowed uneaten *Daphnia* to recirculate through the flow tank. Preparatory trials lasted 3 wk, at the end of which fish that did not readily swim and eat drifting *Daphnia* 15 min after being placed into the flow tank were returned to the Lisha Kill.

Experimental Design

For the experimental trials, I used a population of 50 blacknose dace 1.88 g (0.72–1.92 g) and 5.5 cm (SL) (4.2–6.3 cm) on average. I conducted the experiment with four water velocities (5, 15, 25, and 35 $cm \cdot s^{-1}$), five *Daphnia* delivery rates (0.05, 0.10, 0.15, 0.20, and 0.25 *Daphnia*$\cdot s^{-1}$), and four group sizes (1, 2, 4, and 8 fish). To determine *Daphnia* delivery times, I wrote a computer program that selected a random number between 0 and 1 from a uniform distribution during each second of the food delivery period. If that number equaled or fell below the delivery rate for that trial, the computer signaled the operator to deliver a *Daphnia*. I cross classified these treatments to make 80 different combinations and conducted three replicates of each treatment combination resulting in a total of 240 experimental trials. To prevent any single fish from disproportionally contributing to the data for a particular treatment combination or to the total data set, no fish experienced the same treatment combination twice, and after being used in a trial, a fish was not used again until all 50 had been included in trials.

The acclimation period that preceeded each trial included 15 min with no food followed by the delivery of 20 *Daphnia* at the rate prescribed for the trial. I then recorded 50 observations of *Daphnia* either being eaten by the fish (capture) or passing through the feeding area uncaptured (miss). To ensure that I did not bias the data by overrepresenting uncaptured prey in my observations, I included only those observations in which I saw *Daphnia* enter the feeding area. During the trials, I found that I could follow groups of *Daphnia* in which the first and last individual entered the feeding area no more than about 12 cm apart. In these instances, I recorded the capture/miss of all *Daphnia* in the group. When *Daphnia* in the feeding area were more than 12 cm apart, I recorded the capture/miss of only those *Daphnia* that I observed enter the feeding area.

To prevent interference competition from affecting the results, I removed any fish that overtly and consistently tried to displace others and prevent them from feeding during trials. These "aggressive" fish routinely ignored *Daphnia* drifting past them and clearly were not feeding. Over the course of the experiments, I identified and removed seven aggressive fish. I kept a pool of fish that had undergone the preparation procedure in the laboratory to replace aggressive individuals and fish that died or became ill during the course of the study. The replacement fish were collected at the same time and were approximately the same size as those that they replaced.

Data Analysis

I used a modified stepwise variable selection procedure with nonlinear regression to determine the variables that explained significant proportions of the variance found in the proportion of *Daphnia* that the dace captured. My modification of the

TABLE 1. Mean proportion of *Daphnia* captured (± 1 SE) by the group of foraging blacknose dace; $N = 3$ for each treatment combination.

Group size	Water velocity ($cm \cdot s^{-1}$)	Prey availability ($Daphnia \cdot s^{-1}$)				
		0.05	0.10	0.15	0.20	0.25
1	5	0.87 ± 0.024	0.86 ± 0.036	0.82 ± 0.011	0.92 ± 0.011	0.85 ± 0.005
	15	0.89 ± 0.038	0.88 ± 0.011	0.78 ± 0.047	0.71 ± 0.061	0.81 ± 0.033
	25	0.57 ± 0.036	0.49 ± 0.029	0.57 ± 0.047	0.60 ± 0.043	0.45 ± 0.014
	35	0.03 ± 0.014	0.05 ± 0.029	0.12 ± 0.019	0.06 ± 0.034	0.08 ± 0.042
2	5	0.95 ± 0.014	0.81 ± 0.020	0.85 ± 0.014	0.84 ± 0.016	0.79 ± 0.043
	15	0.92 ± 0.033	0.87 ± 0.063	0.73 ± 0.071	0.92 ± 0.025	0.75 ± 0.061
	25	0.64 ± 0.025	0.39 ± 0.005	0.54 ± 0.043	0.71 ± 0.054	0.59 ± 0.066
	35	0.14 ± 0.019	0.30 ± 0.050	0.16 ± 0.016	0.26 ± 0.071	0.25 ± 0.011
4	5	0.97 ± 0.014	0.96 ± 0.020	0.95 ± 0.014	0.96 ± 0.009	0.96 ± 0.009
	15	0.95 ± 0.024	0.91 ± 0.033	0.92 ± 0.033	0.91 ± 0.014	0.92 ± 0.025
	25	0.61 ± 0.052	0.51 ± 0.061	0.39 ± 0.058	0.44 ± 0.050	0.53 ± 0.020
	35	0.41 ± 0.101	0.45 ± 0.080	0.46 ± 0.028	0.42 ± 0.033	0.52 ± 0.019
8	5	0.97 ± 0.011	0.98 ± 0.001	0.97 ± 0.014	0.96 ± 0.001	0.96 ± 0.019
	15	0.96 ± 0.001	0.98 ± 0.001	0.97 ± 0.011	0.93 ± 0.030	0.96 ± 0.001
	25	0.73 ± 0.020	0.76 ± 0.001	0.76 ± 0.052	0.79 ± 0.069	0.63 ± 0.033
	35	0.46 ± 0.033	0.52 ± 0.055	0.52 ± 0.050	0.46 ± 0.050	0.41 ± 0.082

TABLE 2. Stepwise selection of variables included in the regression describing the proportion of *Daphnia* captured by blacknose dace. Variables considered are water velocity (W), group size (G), and food availability (A). $r^2_{X.YZW}$ represents the residual sum of squares accounted for by the variable X after the effects of the variables Y, Z, and W have been accounted for. See Methods (Data Analysis) for description of variable selection procedure.

Factor	Factor and exponent	r^2	$r^2_{X.Y}$	$r^2_{X.WG}$	$r^2_{X.WGW \cdot G}$	F
Main effects						
W	$W^{1.9556}$	−0.8754[a]	—	—	—	780.64
G	$G^{0.9761}$	0.2652	0.5486[b]	—	—	102.02
A	$A^{4.3132}$	−0.0144	0.0167	−0.0983	−0.0830	1.6287[d]
Interactions						
$W \cdot G$	$(WG)^{0.8375}$	−0.1952	0.3345	0.1214[c]	—	3.53
$W \cdot A$	$(WA)^{0.4637}$	−0.0144	0.0167	−0.0983	−0.0830	1.6287[d]
$G \cdot A$	$(GA)^{0.2630}$	−0.0144	0.0167	−0.0983	−0.0830	1.6287[d]
$W \cdot G \cdot A$	$(wga)^{0.4208}$	−0.0978	0.1550	−0.0983	−0.0517	0.6290[d]

[a]df = 1,238, $p << 0.001$.
[b]df = 2,237, $p << 0.001$.
[c]df = 3,236, $p < 0.05$.
[d]df = 4,235, $p > 0.10$.

standard procedure was to first determine the value of exponents for each variable with SPSS nonlinear regression prior to performing stepwise selection. Thus, the variables used in stepwise selection were of the form X^{α} rather than X and for interaction terms were of the form $(X_1 X_2)^{\alpha}$ rather than $(X_1 X_2)$. I then used standard, forward stepwise variable selection to obtain partial correlation ($r^2_{X.YZW}$) and F values for each variable ($F \geq 3.0$ to include) and to determine the regression equation describing blacknose dace foraging efficiency. This procedure produced a regression equation that includes only those variables that explain a significant proportion of the variance observed in the proportion of *Daphnia* captured by the fish.

Results

The proportion of *Daphnia* that the blacknose dace captured in a trial ranged from 0.0 to 1.0 (Table 1). Water velocity (W), group size (G), and the water velocity by group size interaction ($W \cdot G$) were the only variables that explained significant proportions of the variance in the proportion of *Daphnia* captured. Prey availability (A) and the interactions that included prey availability ($W \cdot A$, $G \cdot A$, and $W \cdot G \cdot A$) neither explained significant proportions of the variance in the proportion of *Daphnia* captured nor significantly increased the predictive power of the nonlinear regression (Table 2). Therefore, these factors were not included in the regression equation.

The regression relating the proportion of *Daphnia* captured (P) to water velocity, group size, and their interaction resulting from stepwise variable selection explained a large amount of the variance observed in fish's foraging efficiency ($R^2 = 0.853$, MSE = 0.0131, df = 236) (Fig. 3):

$$(1) \quad P = 0.893 - 0.00076W^{1.956} + 0.0046G^{0.976} + 0.0030(WG)^{0.838}.$$

Increases in water velocity resulted in a pronounced decrease in the proportion of drift food the fish captured. Increases in the number of fish in the foraging group increased the proportion of food the group captured, but only at fast water velocities. At slow water velocities, solitary fish captured over 80%

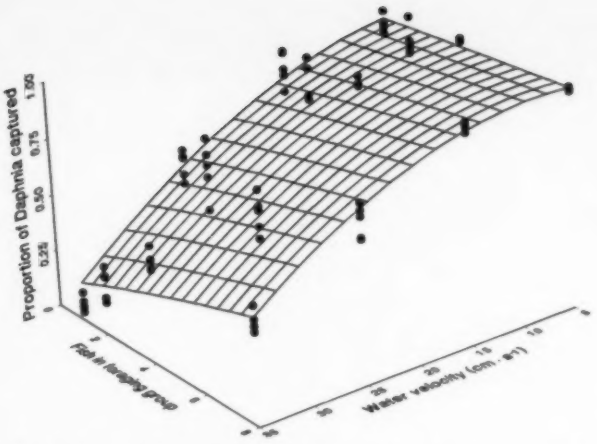

FIG. 3. Effects of water velocity and group size on the proportion of food items captured by the foraging group. Each point represents the average proportion captured over three trials. Multiple points at the same water velocity – group size intersection represent data collected at different prey arrival rates.

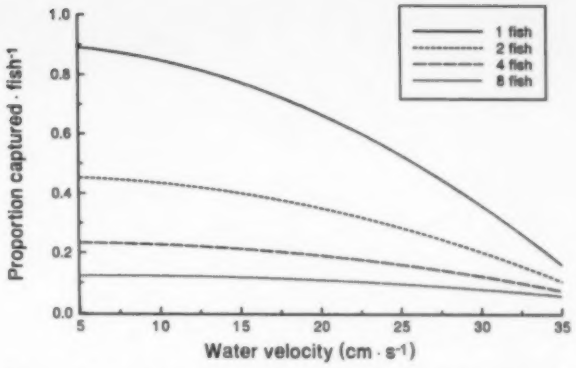

FIG. 4. Effects of water velocity and group size on the proportion of food captured by individual fish derived from regression equation (1).

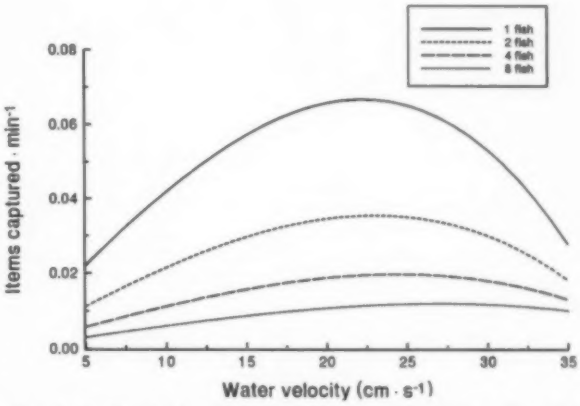

FIG. 5. Predicted relationship between per capita foraging rates and water velocity and group size, assuming a uniform distribution of food throughout the water column at a density of 0.82 items·1000 L^{-1} and a search area for individual fish of 100 cm^2. Relationship derived from equation (1).

of the available food; therefore, increases in group size when water velocity was slow could not allow the group to capture considerably more prey and apparently resulted in individuals obtaining less food per capita. These results indicate that water velocity played an important role in determining both the proportion of *Daphnia* the fish captured and the degree to which competition affected per capita food intake.

In collecting and analyzing these data, I assumed that all individuals foraged equally, which was not the case. Some of the fish captured a higher proportion of the *Daphnia* than did others. Fish that captured a greater than average proportion of *Daphnia* were more active and "quicker" than the average fish. I frequently observed these successful individuals capturing *Daphnia* that had drifted past them and/or drifted down the opposite side of the feeding area, more than 20 cm (four body lengths) away. Highly successful fish did not have a consistent size advantage over less successful fish.

Discussion

From the regression describing the proportion of *Daphnia* captured by a group of blacknose dace, I have inferred the effects of changes in water velocity and group size on per capita foraging efficiency (Fig. 4). I derived the effects of these two factors on the per capita proportion of *Daphnia* captured by dividing the regression for the proportion of items the foraging group captured (equation (1)) by the number of individuals in the group. At small group sizes, increases in water velocity greatly reduced the proportion of food items individual fish captured. Similarly, at slow water velocities, an increase in the group size greatly reduced the proportion of food items an individual captured. However, if either water velocity was fast or the number of fish in the group was high, then the per capita proportion of food captured was low regardless of the level of the other factor. One would expect, then, that drift-feeding fish would not forage in groups when foraging in areas with fast-moving water, or that they would avoid fast-moving water when foraging in groups. Situations in which minnows form groups while foraging in areas with fast water may represent circumstances in which grouping provides a reduction in an individual's predation risk; such circumstances could provide an energetic advantage only if food were so abundant that competition did not affect per capita intake.

To determine the effect of water velocity and group size changes on the per capita intake rate of the fish (items captured per minute), I assumed that fish could capture prey that passes within a 100-cm^2 cross section of the stream and that prey had a uniform distribution throughout the stream of 0.82 items·1000 L^{-1} (J.A. Tyler, unpublished data for the Lisha Kill, August 1988). I inferred the effect of changes in water velocity and group size on per capita intake rate using the formula

$$(2) \quad I = D \cdot C \cdot W \cdot \frac{P}{G}$$

where I = per capita intake rate (items captured per minute), D = density of prey items (items per cubic centimetre), and C = cross-sectional area of stream (square centimetre). Per capita intake rate exhibits a maximum at water velocities between 22 and 27 cm·s^{-1} for group sizes ranging from one to eight fish (Fig. 5). In addition, per capita intake rate is far more

sensitive to differences in water velocity at small group sizes than it is at large groups sizes. If we define optimal water velocity as that speed at which individuals capture the most prey per minute, then when competition for food is low or nonexistent (small group sizes), fish that forage in areas of nonoptimal water velocities experience notably lower intake rates than those fish in areas of optimal water velocity. High levels of competition (large group sizes) cause the relationship between water velocity and per capita intake rate to become nearly flat. Thus, when competition for food is high, the advantage gained from selecting foraging areas of optimal water velocity is relatively small. Regardless, at all levels of competition, fish capture more food items at intermediate water velocities than they capture at high water velocities (Fig. 5).

The discussion of per capita foraging above should be taken strictly as a characterization of general trends. I have extrapolated the regression equation (1), developed for foraging groups, to gain insight into individual foraging success. However, because individuals exhibit different foraging abilities and because I cannot estimate the size of these differences, the general inferences I make about individual foraging success may break down when applied to the foraging of particular individuals.

The relationship between per capita intake and water velocity only partly supports the common view that sites in fast water are good because of the high prey encounter rates they provide fish (Chapman and Bjornn 1969; Symons 1976; Li and Brocksen 1977; Wańkowski and Thorpe 1979; Fausch and White 1981; Fausch 1984). Per capita intake rate increases as water velocity increases up to 24 $cm \cdot s^{-1}$ (Fig. 5). This apparently results from the increased prey encounter rates that fish gain as water velocity increases. Over this range of water velocities, solitary fish capture at least half of the available prey (Fig. 4). As water velocity increases over 24 $cm \cdot s^{-1}$, per capita intake drops. The relationship between per capita intake rate and water velocity for blacknose dace is qualitatively similar to the relationship that Grant and Noakes (1987, 1988) found between foraging attempts per minute and water velocity for brook trout. The drop in per capita intake at fast water velocities could have a number of causes: (1) fish may have difficulty catching fast-moving prey before they are carried beyond the fish's reach (Hughes and Dill 1990), (2) fish may attempt to catch fewer prey because each attempt is energetically expensive (Godin and Rangeley 1989), or (3) fish may detect fewer prey when they are moving fast. Regardless of the cause, these results along with those of Grant and Noakes (1987, 1988), Hill (1989), and Hughes and Dill (1990) add to the growing evidence that the advantages of foraging in fast water do not always outweigh the disadvantages.

The work of Wilzbach et al. (1986) and the results presented here demonstrate that not only do habitat characteristics affect fish foraging efficiency by themselves, but their effects may interact significantly. This experiment shows that although water velocity and group size each affect dace capture efficiency, because they have a significant interaction, the effects of one factor cannot be fully understood without accounting for the effects of the other factor. In addition, the strength of effect from one habitat characteristic may override the effects of other habitat characteristics. For example, Wilzbach et al. (1986) found that the amount of surface light and substrate crevices affected trout foraging, but the effect of logging overwhelmed the effects of the other two factors.

Hill (1989) and Hughes and Dill (1990) showed that microhabitat selection models that include a quantitative description of individual foraging success more accurately predict fish microhabitat use than models that do not include such a description. The factors studied here represent only a small portion of the factors that could have important effects on fish foraging efficiency. Other potentially important factors include light level, cover, substrate composition (Wilzbach 1985; Wilzbach et al. 1986), turbidity, and the type of food items found in the drift (G.D. Grossman, University of Georgia, personal communication). The results of this study suggest that future research should be conducted to produce quantitative descriptions of the relationship between foraging efficiency and the characteristics of the habitats fish inhabit.

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Stock Discrimination Using Otolith Shape Analysis

Steven E. Campana

Department of Fisheries and Oceans, Marine Fish Division, Bedford Institute of Oceanography, P.O. Box 1006, Dartmouth, N.S., Canada B2Y 4A2

and John M. Casselman

Ontario Ministry of Natural Resources, Fisheries Research, Glenora Fisheries Station, R.R. 4, Picton, Ont., Canada K0K 2T0

Campana, S.E., and J.M. Casselman. 1993. Stock discrimination using otolith shape analysis. Can. J. Fish. Aquat. Sci. 50: 1062–1083.

Otolith shape has long been known to be species specific, but recent reports have pointed to its value as an indicator of stock identity. To test this hypothesis, all three pairs of otoliths were sampled from 2349 Atlantic cod (*Gadus morhua*) collected on spawning grounds throughout the northwest Atlantic. Otolith shape was determined with Fourier analysis and combined with measures of otolith area and perimeter. There were highly significant differences in otolith shape among most of the cod samples, but shape also differed among ages, sexes, and year-classes. The sagittal otoliths (largest pair) provided slightly better stock discrimination than did the lapillar or asteriscal otoliths. The first discriminant function was highly correlated with both fish and otolith growth rate, indicating that stock discrimination improved as the difference in stock-specific growth rate increased. Reevaluation of published studies on other species indicated that growth rate contributes more variation to regional differences in otolith shape than does stock origin. Differences in otolith shape among ages, sexes, and year-classes were also attributable to growth rate differences. To the extent that growth rates vary more between than within stocks, otolith shape analysis can provide an easily determined measure of stock identity.

On sait depuis longtemps que la forme des otolithes est particulière à une espèce, mais des travaux récents ont souligné sa valeur comme indicateur de l'identité d'un stock. Pour tester cette hypothèse, on a prélevé les trois paires d'otolithes chez 2 349 morues franches (*Gadus morhua*) capturées sur les frayères dans tout l'Atlantique nord-ouest. La forme des otolithes a été déterminée par une analyse de Fourier, et combinée à des mesures de la superficie et du périmètre des otolithes. On a noté des différences hautement significatives dans la forme parmi la plupart des échantillons de morue, mais la forme différait aussi entre les âges, les sexes et les classes annuelles. Les sagitta (la paire la plus grosse) permettaient une discrimination des stocks légèrement meilleure que les lapillus ou les asteriscus. La première fonction discriminante était fortement corrélée au taux de croissance du poisson et de l'otolithe, ce qui indique que la discrimination des stocks s'améliorait à mesure qu'augmentait la différence dans le taux de croissance de chaque stock. Le réexamen des travaux publiés sur d'autres espèces indique que le taux de croissance fait varier davantage les différences régionales dans la forme des otolithes que l'origine du stock. Les différences dans la forme des otolithes entre les âges, les sexes et les classes annuelles étaient aussi attribuables à des différences dans le taux de croissance. Dans la mesure où les taux de croissance varient davantage entre les stocks qu'à l'intérieur d'un stock, l'analyse de la forme des otolithes peut constituer une méthode facile pour déterminer l'identité des stocks.

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Stock discrimination forms the basis for much of fisheries management in the northwest Atlantic. Measures of growth, survival, and reproductive success all assume that a single population is being monitored. Where such measures are confounded by population mixing, studies of fish biology, population dynamics, and most estimates of yield can be invalidated. In principle, genetic differentiation should form the basis for any inferences concerning population distinction. However, analyses based on protein electrophoresis (Cross and Payne 1978; Mort et al. 1985; Grant et al. 1987; Mulligan et al. 1988) and mitochondrial DNA (Smith et al. 1989; Carr and Marshall 1991; Dahle 1991) have, in general, been unsuccessful in differentiating among marine populations. Under the assumption that stocks loosely represent populations, tagging (Wise 1963), morphometrics (Thorpe 1976), meristics (Blouw et al. 1988), parasite loads (Scott and Martin 1957), ichthyoplankton surveys (O'Boyle et al. 1984), immunological characteristics (Schill and Dorazio 1990), and other approaches have all been used to confirm the presence of multiple stocks, but none has provided a reliable indicator of stock identity. Indeed, there appear to be few, if any, markers which can be used to differentiate among all populations of any marine fish species.

The shape of the otolith would appear to be an ideal natural marker for fish populations. Otolith shape is markedly species specific (Morrow 1976; Gaemers 1984; L'Abée-Lund 1988) and less variable in growth than fish growth, presumably due to the dual function of the otolith as an organ of equilibrium and hearing. Otoliths grow throughout the life of the fish and, unlike scales and bone, are metabolically inert; once deposited, otolith material is unlikely to be resorbed or altered (Campana and Neilson 1985; Casselman 1987). Therefore, otoliths remain unaffected by the short-term changes in fish condition (e.g., starvation) which can confound body morphometrics. Since otoliths are composed of calcium carbonate, otolith shape is

TABLE 1. Sample collection.

Sample No.	Area (NAFO division)	Site acronym	Date	Latitude	Longitude	Sample size
1	Banquereau Bank (4Vs)	Banq1	Mar. 7, 1986	44°18′	59°02′	122
2	Banquereau Bank (4Vs)	Banq2	Mar. 22, 1986	44°41′	58°57′	129
3	Browns Bank (4X)	Brown88	Feb. 29, 1988	42°45′	66°10′	104
4	Browns Bank (4X)	Browns	Feb. 19, 1986	42°45′	66°10′	141
5	Cape Cod (5Zeg)	Capecod5Ze	Jan. 30, 1986	41°40′	69°30′	79
6	Cheticamp (4T)	Cheticamp	May 26, 1986	46°20′	61°20′	150
7	Fundy Rip (4X)	Fundyrip	Mar. 6, 1986	44°10′	66°30′	145
8	Gabarus (4Vn)	Gabarus	May 27, 1986	45°40′	59°45′	150
9	Georges Bank (5Ze)	George5Ze	Mar. 4, 1986	42°00′	66°00′	20
10	Georges Bank (5Zj)	George5Zj	Apr. 16, 1986	42°10′	67°05′	150
11	Grand Manan (4Xs)	Grandmanan	Mar. 13, 1986	44°28′	66°32′	109
12	Green Island (4Wd)	Green4Wd	Apr. 29, 1986	45°25′	60°45′	140
13	Iceland (—)	Iceland	May 6, 1986	65°15′	23°30′	65
14	Larkinridge (5Yb, 4Xq)	Larkinridge	Jan. 28, 1986	43°50′	67°30′	145
15	Newfoundland (3Ld)	Nfld3ld	Apr. 24, 1986	49°12′	50°30′	199
16	Newfoundland (3Ode)	Nfld3od	May 26–28, 1986	44°03′	52°05′	150
17	Stellwagen Bank (5Ye)	Stellwagen	Jan. 31, 1986	42°15′	70°20′	109
18	Western Bank (4W)	Western	Mar. 3, 1986	43°19′–43°36′	60°03′–61°21′	119
19	Western Bank (4W)	Western88	Mar. 23, 1988	44°00′	61°35′	123

unaffected by the mode of fish preservation (excluding acidic preservatives, which promote dissolution). As is the case with morphometrics, meristics, and all other stock identification procedures except DNA sequencing, the use of otolith shape to infer stock identity does not distinguish between genetic and environmental differences. Nevertheless, as long as such differences exist, otolith shape should vary among populations as long as they remain at least partially segregated.

Otolith appearance and shape often vary geographically within a species, although there are mixed reports concerning the potential for stock discrimination. In some cases, reports of stock identification based on otoliths appear to reflect environmental differences among regions; environmental effects are almost certainly the cause of geographic variations in the shape or appearance of the otolith nucleus (Messieh 1972; Godø 1984; Neilson et al. 1985; Dawson 1991), otolith annuli (Rollefsen 1933, cited in Reisagg and Jørstad 1984; Rauck 1974; Godø 1984), and variations in the ratio of otolith size to fish size (Templeman and Squires 1956; Rojo 1977). However, measures of otolith shape based on ratios of otolith radii (Maceina and Murphy 1989) and in particular Fourier analysis (Williams 1980; Casselman et al. 1981; Bird et al. 1986; Castonguay et al. 1991) have had some success in distinguishing among stocks and have been assumed to be based, at least in part, on genetic differences. Nevertheless, there have been consistent reports of otolith shape variations among ages, sexes, and year-classes within a stock (Casselman et al. 1981; Bird et al. 1986; L'Abée-Lund 1988; Castonguay et al. 1991), leaving some doubt as to the overall utility of the technique.

The objectives of this study were to provide an in-depth appraisal of the value of otolith shape analysis for stock identification. Since the previous studies had examined relatively few samples, the current study was designed to examine a large number of fish, spread over a broad geographic area and a large number of putative stocks. The Atlantic cod (*Gadus morhua*) stock complex in the northwest Atlantic Ocean was selected as the test species, in part due to its distribution and number of resident stocks, and in part because of the background knowledge of stock structure already available for this species (McKenzie 1934, 1956; Templeman 1962; Wise 1963; Cross and Payne 1978; Lear and Wells 1984; Mork et al. 1985; Lear 1986; Dahle 1991). Additional factors evaluated in this study were age, sex, year-class effects, and the relative contributions of genetic and environmental effects to otolith shape variation. Since all previous studies examined only the sagittal (largest) otolith pair, we also examined the additional stock structure information present in the shape of the other two otolith pairs. Fourier analysis was selected as the most objective and powerful of the shape analysis techniques. However, careful attention was also given to the apparent shape differences that arise through selection of the nucleus (rather than the centroid) as the centre of the otolith, and the influence of fish length on the Fourier variables.

Materials and Methods

Sampling

The sampling program was designed to catch fish of known stock through capture on the spawning ground in spawning condition. It is generally assumed that stock mixing is minimal at the time of spawning and that seasonal feeding migrations begin long after spawning has been completed (Templeman 1962). While not all of the cod used in this study were in spawning condition, most were preparing to spawn or had just spawned. Accordingly, we believe that our samples are reasonable representations of a number of discrete spawning stocks.

Cod were either collected at sea aboard research vessels using otter trawl gear or sampled from commercial catches where the fishing location was unambiguous. Most samples consisted of fish from two or more tows. Samples were restricted to fish in the size range of 45–85 cm fork length, so as to restrict the analysis to sexually mature individuals and to reduce variability caused by size-related effects. A total of 2349 fish were collected from 19 sites along the eastern coast of Canada, the northeastern coast of the United States, and several miles off of the western coast of Iceland (Table 1; Fig. 1). Most of the putative cod stocks in the northwestern Atlantic Ocean were sampled. All samples were collected in 1986, with the exception

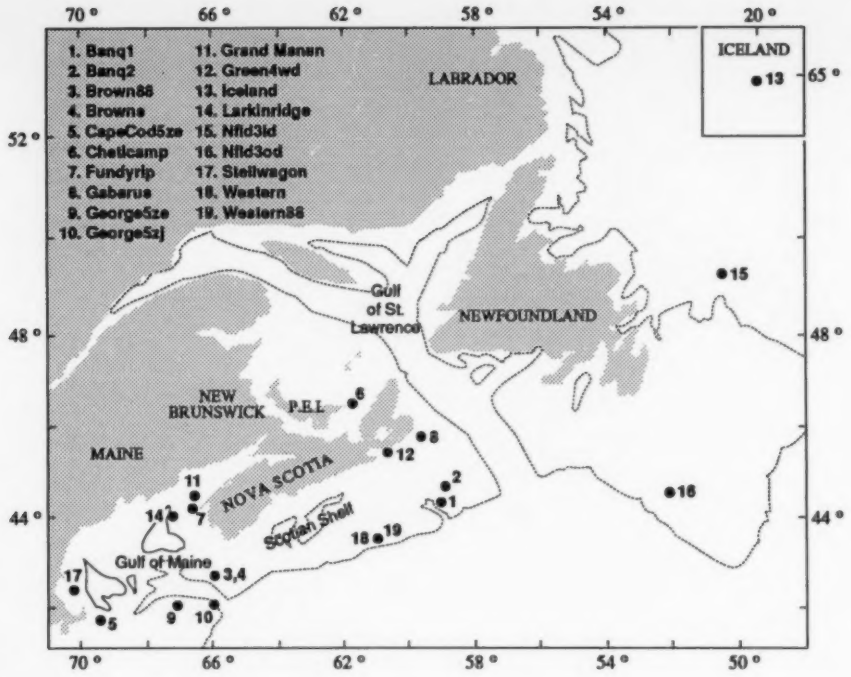

FIG. 1. Map of the study area and sampling sites. The sample from Iceland was collected several miles off the western coast of Iceland. The 200-m contour is shown.

of two sites (Browns Bank and Western Bank), which were also sampled in 1988 to test for year effects within a site. Sampling dates varied across several months because of the tendency for cold-water stocks to spawn later than warm-water stocks.

Immediately after capture, fork length, head length (from the tip of the snout to the posterior end of the preoperculum), sex, and state of sexual maturity were recorded. The head was then severed, labelled, and frozen for subsequent otolith removal in the laboratory. All three otolith pairs (sagittae, lapilli, and asterisci) were removed from each fish, cleansed of adhering tissue, and stored dry in vials until they could be examined further. Broken and crystalline otoliths were discarded (<1% of the sample).

Since the lapillar and asteriscal otolith pairs are not normally collected from fish, their location relative to the brain and the sagittae is reported here. Cod lapilli were located well anterior and dorsal to the sagittae, lateral to the forebrain. This small pair of otoliths was generally removed while still within the endolymphatic canals (inner ear system), which lie in a lateral cranial invagination at the location of the lapilli. Asterisci were also often removed in association with endolymphatic canals, but in a location just posterior and immediately adjacent to the sagittae. A sagitta and asteriscus could often be removed together with forceps if the endolymph around the sagitta was gripped at the same time as the sagitta.

Otolith Data

The shape of each of the six otoliths from each fish was analyzed as a two-dimensional projection (outline), as is common practice. After differentiating the left and right otoliths of each otolith pair, otolith shape was quantified in a two-step procedure consisting of (1) digitization of the otolith shape and (2) conversion of the shape coordinates to Fourier components.

In the first step of the procedure, each otolith was oriented in a consistent manner on a dissecting microscope stage (Fig. 2); sagittae were oriented with the sulcus up, the pyramid-shaped lapilli were placed with the large, rough surface down, and the asterisci were oriented with the concave side down. All otolith measurements and examinations were carried out at microscopic magnifications of 3.2× (sagittae) or 8× (lapilli and asterisci) via an image analysis system (Campana 1987). Using external otolith morphology for guidance, the coordinates of the otolith nucleus were digitized. The image was then converted to a binary image and the area and perimeter of the otolith calculated using standard image analysis procedures. The X–Y coordinates of the otolith edge were determined using an edge-following subroutine; as a result, errors due to tracing on a digitizer pad were avoided. To provide a common starting point for the otolith edge coordinates, the position of a standard landmark was digitized on each of the otolith images by the operator before the edge detection procedure was started (Fig. 2). While the selection of a given landmark was arbitrary, use of standard landmarks ensured that the phase angles of the subsequent Fourier analysis were interpretable in the same way across all otoliths of a given type. The landmarks used were as follows: sagittae, tip of rostrum; lapilli, acute vertex at the confluence of the rough and smooth surfaces; asterisci, tip of the largest lobe. Typically, this stage of the shape analysis procedure resulted in 700–1000 X–Y coordinate pairs for the sagittae, 300–400 for the asterisci, and 200–300 for the lapilli. The length of the long axis of the otolith was also calculated from these data.

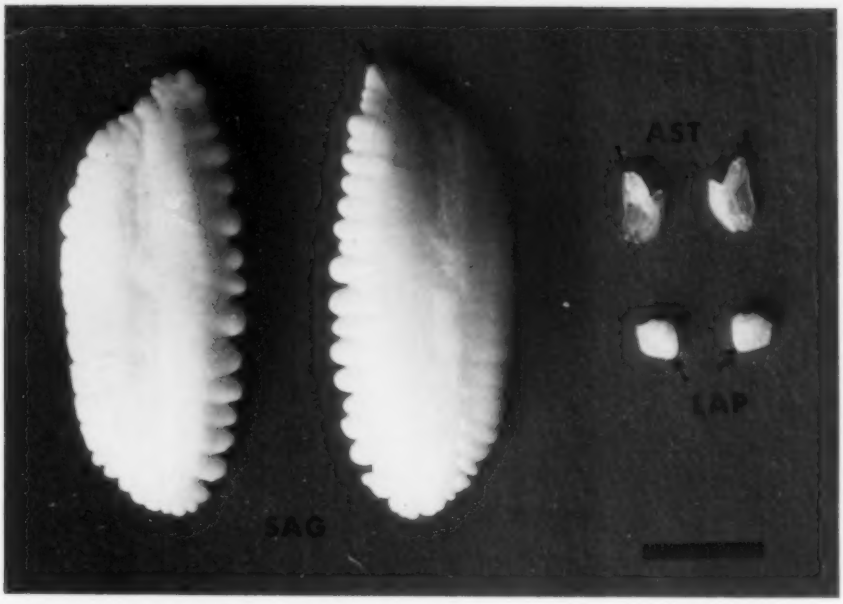

FIG. 2. Photograph of the three otolith pairs (SAG = sagittae; LAP = lapilli; AST = asterisci) from an 85-cm cod. Each otolith pair is presented with the left-hand otolith on the left side. The landmark used to begin the edge-following procedure is indicated by an arrow. Bar = 5 mm.

The second stage of the shape analysis procedure involved the interpretation of the otolith contour data in terms of Fourier components. Fourier analysis has been described in detail elsewhere (Younker and Ehrlich 1977; Full and Ehrlich 1982; Bird et al. 1986) and will not be repeated here. Mathematically, the length of the otolith radius R at angle θ is described by

$$(1) \qquad R(\theta) = A_0 + \sum_{n=1}^{\infty} A_n \cos(n\theta - \phi_n)$$

where θ is the polar angle measured from the landmark on the contour, A_0 is the mean radius length (the amplitude of the 0th harmonic), A_n is the amplitude of the nth harmonic, and ϕ_n is the phase angle of the nth harmonic (Bird et al. 1986).

Conceptually, Fourier analysis can be considered to be a means of describing a shape in terms of cosine waves. A series of radii are drawn at equal angular intervals from some central location within the contour to the corresponding coordinates along the contour. The shape is then opened, or unrolled, from a specified landmark, leaving the radii as a sequence of lines of variable length. A single cosine wave is fitted to the data, so as to mimic as closely as possible the undulation of the top of the unrolled radii. This cosine wave can be described as an amplitude (height) and phase angle (position along the unrolled contour). Since cosine waves are additive, a second cosine wave of different amplitude and phase angle can then be added to the first, thus explaining more of the observed shape variation. Similarly, subsequent cosine waves (also termed harmonics) can be added to the first two, until the observed shape has been almost fully described. Addition of successive harmonics adds increasing detail to the description of the shape. The shape described by the nth harmonic represents that of a n-leafed clover. There is no limit to the number of harmonics that can be used to describe and/or reconstruct a shape. In practice, however, it is best to describe the shape in as few terms as possible so as to facilitate the statistical analysis of the amplitude and phase variables which follows. Figure 3 demonstrates the sequential addition of harmonic terms which would be used to describe the shape of a typical sagittal otolith.

In this study, the Fourier analysis procedure of Jarvis et al. (1978), modified by J.M. Casselman and K. Scott (unpublished), was used to convert the otolith contour coordinates ($n = 72$, corresponding to an angular interval of 5°) into Fourier coefficients. Fourier coefficients were calculated in two ways: otolith contours were unrolled counterclockwise around both the digitized nucleus and the calculated centroid, starting from the digitized landmark. Twenty harmonics were calculated for each otolith. However, since 95–99% of the shape variation was described by the first 10 harmonics, only the latter were included in the statistical analysis. Both the raw (unstandardized) and the standardized (amplitude divided by the mean radial length) amplitudes were available for statistical analysis, as were the corresponding phase angles.

After the shape data had been collected, the sagittae were sectioned and aged according to established procedures (J. Hunt, St. Andrews Biological Station, St. Andrews, N.B. E0G 2X0, personal communication). None of the fish were of known age, so ageing accuracy could not be determined. However, the age reader (R. Robicheau) was one of the most experienced cod otolith readers in the Maritimes. In order to evaluate ageing precision, a random subsample (10%) of each of the sagittal samples was blind-coded and reread by the same person approximately 6 mo after the initial reading. Ageing precision was good for all stocks, with coefficients of variation (Chang 1982) ranging from 0 to 9% and a mean of 2.0%.

The mean growth rate of each fish was calculated as the fork length divided by the age. The mean otolith growth rate of each fish was the length of the otolith's long axis divided by the age.

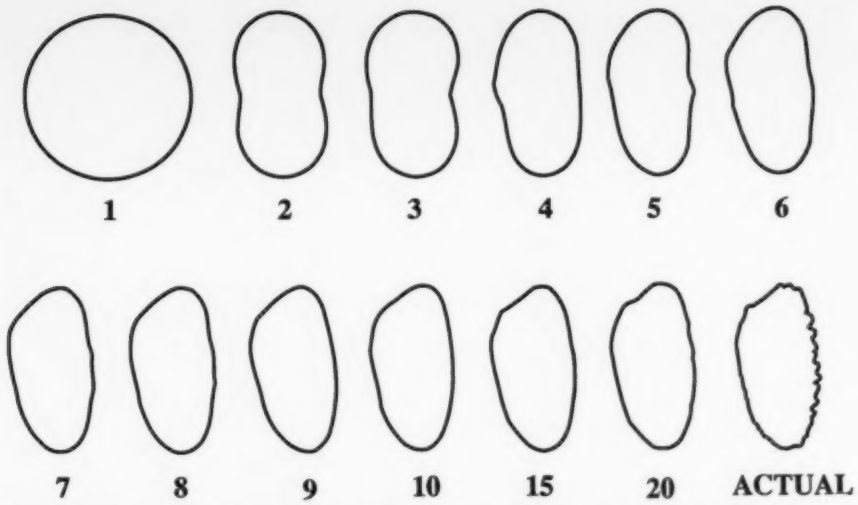

FIG. 3. Reconstruction of a typical sagitta from its Fourier variables, using the centroid as the otolith centre. The number within each shape represents the number of harmonics used to prepare the shape. The actual digitized shape is presented last. Note that the rough outline is reconstructed relatively accurately with only the first six harmonics, but that the scalloped edges require more than 20 harmonics.

Statistical Analysis

The intent of the statistical design was to test the classification success of a series of discriminant functions used to predict cod stock identity. Stock identity was presumed to be known, based on the collection of near-spawning individuals on or near a primary spawning ground in the middle of the spawning season. Discriminant functions were developed using the Fourier coefficients (which by definition are orthogonal), otolith area, and otolith perimeter and then tested for classification success against otoliths of known sampling site which were not used in the preparation of the discriminant functions. The latter will be referred to as the test sample.

All variables entered into the discriminant analysis were first examined for normality, and if necessary, natural log (ln) or square root transformed. The phase angle data were, of course, circularly distributed between 0 and 360° and thus were often bimodally distributed if the median value was near 0 or 360. There was no obvious method by which these data could be normalized (Batschelet 1981). We attempted to induce a unimodal distribution in each bimodal phase variable by adding 360° to all observations that appeared to be more closely associated with the lower mode than the upper mode. However, selection of the threshold value separating the two modes was based only on visual inspection of the data distribution, and in any case, often resulted in a nonnormal, unimodal distribution that could not be readily transformed. Accordingly, we have given relatively little weight to the phase variables in this study.

Since all variables except the phase angles were correlated with otolith length and fish length, significant intersample differences in otolith shape could have resulted artifactually from differences in length frequency among samples. Accordingly, all otoliths were standardized to a common size by removing the common, within-group slope of otolith length on all variables. The effect of otolith length, rather than fish length, was removed from the variables, since fish length measurements were not available for two of the samples, and more importantly, otolith length was unaffected by intersample differences in preservation, shrinkage, and distortion. Bartlett's test for homogeneity of variance indicated that there was often significant heterogeneity of variance among samples in the ANOCOVA model. However, given the large sample sizes, the conservative nature of Bartlett's test, and the random distribution of the model residuals, we do not believe the model results were unduly influenced by the intersample differences in variance. Similarly, the finding that a nested ANOCOVA explained significantly more of the variance in some variables than did the common slope model was considered to be more an artifact of the large sample sizes than real differences. Discriminant analyses based on data adjusted for otolith length using nested slopes performed poorly in classifying fish not used in the preparation of the discriminant functions. A second series of analyses was conducted using the standardized amplitudes (amplitudes divided by the mean radial length); the effect of otolith length was not explicitly removed from these variables.

Results

Age and Length Composition

Despite having restricted the sample collections to fish between the lengths of 45–85 cm, there were obvious differences in length frequency among sample sites (Fig. 4). Modal lengths for the southern samples were less than 60 cm, while those for the Newfoundland and Iceland samples were up to 20 cm longer. The distribution of age frequencies among samples also differed substantially (Fig. 5), although not necessarily in parallel with the length frequencies. Cod collected in the Gulf of Maine region averaged 2–5 yr old, while those in more northerly waters averaged 6–8 yr old and some reached 14 yr. Comparisons of growth rate among sample sites indicated that cod were fastest growing in the Gulf of Maine, of intermediate growth rate on the outer Scotian Shelf, southern Newfoundland, and Iceland, and slow growing in the Gulf of St. Lawrence and its approaches. There was more than a threefold difference in mean annual growth rates between cod in the Gulf of Maine and those in the Gulf of St. Lawrence.

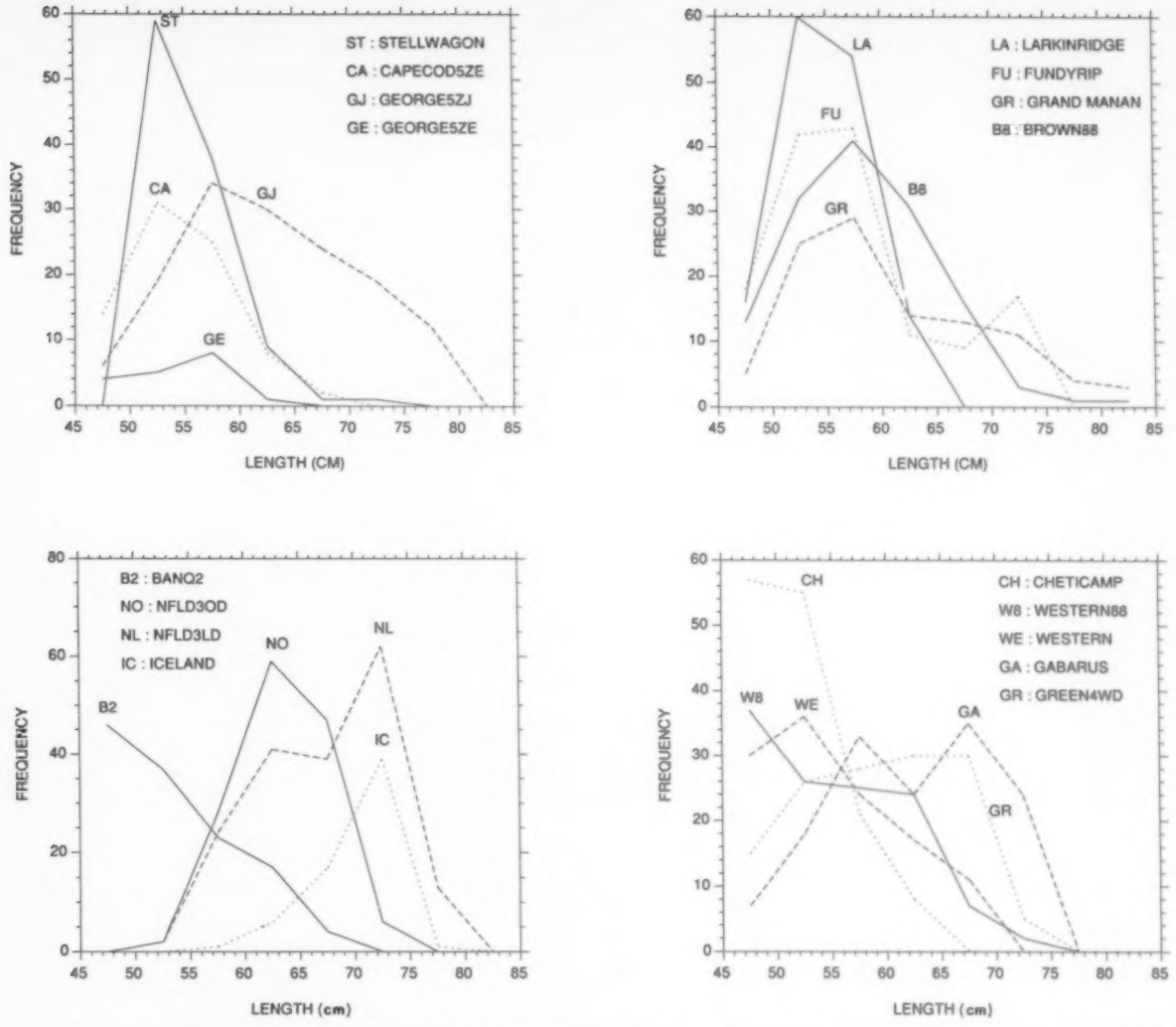

FIG. 4. Length frequencies of cod by sample collection site. Samples were restricted to a length range of 45–85 cm. All fish lengths are presented, with the exception of Browns and Banq1, for which only heads were collected.

Fourier Analysis

Visual examination of the otoliths from each region indicated that otolith shape varied to a different extent among the three otolith types. Lapilli appeared to be most consistent in shape, while asterisci were highly variable, even in a comparison of left versus right from the same fish. In general, otolith size appeared to be more consistent within a fish than otolith shape.

Fourier analysis indicated that more than 99.9% of the otolith shape variability could be summarized by 20 harmonics. The first 10 harmonics explained an average of 99.0, 97.2, and 99.5% in the lapilli, asterisci, and sagittae, respectively; since the distribution of phase angles started to become random after the 10th harmonic, the higher harmonics contained relatively little systematic shape variation and were not included in the subsequent analysis.

The harmonics which explained most of the otolith shape variance were relatively consistent across sample sites, but very different across otolith types (Table 2). The choice of otolith centre (nucleus versus centroid) used in the Fourier analysis was also influential, although by design, centroid-based Fourier calculations discount the influence of the first harmonic to near zero. When the nucleus was used as the centre, over 80% of the variance in lapillar shape was explained by the first harmonic, while the third harmonic explained most of the remaining variance. Fourier analysis with the centroid as the otolith centre resulted in harmonics 2–5 being most important. The more variable asteriscal shapes required six harmonics to explain most of the variation, while the sagittae required five. For all otolith types, each harmonic after the eighth explained less than 1% of the overall shape.

Comparison of the amount of shape variability within and among fish based on the Fourier coefficients confirmed visual impressions that shape was quite variable. Coefficients of variation (CV) for the Fourier amplitudes were 1.5–4.0 times

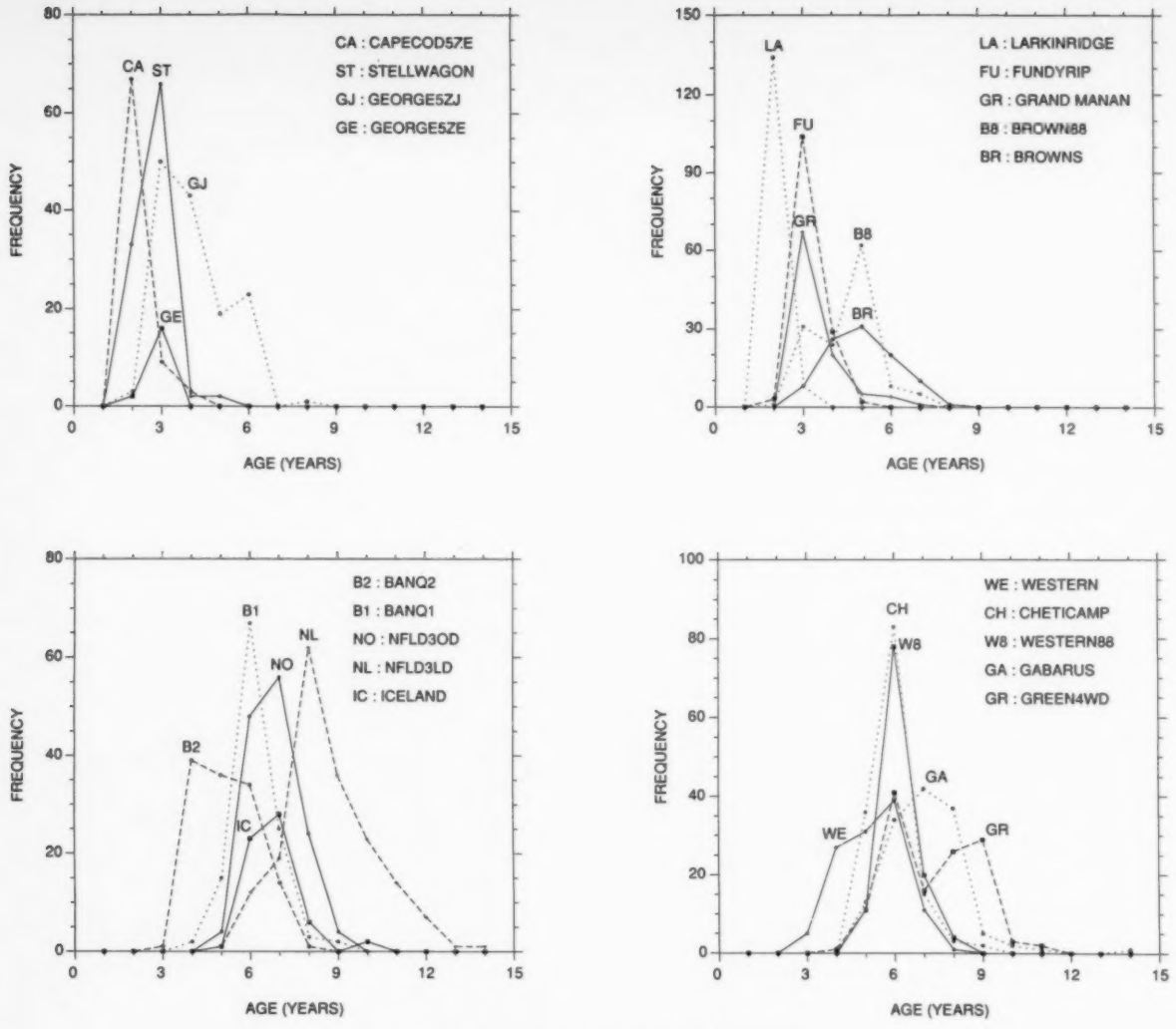

FIG. 5. Age frequencies of cod by sample collection site.

higher among fish of a given sample than between left and right otoliths of the same fish. Otolith area and perimeter CVs were 4–8 times higher among fish than within otolith pairs. Thus, otolith shapes and in particular otolith size were more consistent between the left and right otoliths of a given otolith type than among fish. Comparisons among otolith types within a given fish indicated that asteriscii were most variable and sagittae least variable. There were no significant CV differences among otolith types across fish.

Reconstruction of the mean otolith shape (using the mean of the within-sample, centroid-based Fourier variables) for five representative samples demonstrated that regional shape differences were subtle but discernable (Fig. 6). Lapillar and asteriscal shapes were faithfully replicated by the reconstruction. The overall outline of the sagittae was also well reconstructed; however, the lateral scalloping so characteristic of cod sagittae was absent, since it represented fine detail not fully described by the first 10, or even 20, harmonics (Fig. 3).

Age, Length, and Sex Effects on the Shape Variables

Correlation tables prepared for each of the otolith types demonstrated that few of the shape variables were significantly correlated with each other. With respect to the sagittae, only the otolith perimeter (ln transformed) and otolith area (ln-transformed square root of the area) were highly correlated in either the centroid-as-centre or nucleus-as-centre analysis ($r = 0.93$ in both analyses). Most of this correlation was due to a fish length effect which, when removed, eliminated much of the correlation. Hence, both variables were left in the analysis. The distance from the nucleus to the centroid (CFOCUS) was highly correlated with the first amplitude ($r = 0.86$); therefore, it was removed from the analysis. While there was some tendency for the first amplitude to be correlated with the next one to four amplitudes, this was not considered to be a serious problem. None of the amplitudes were significantly correlated with the corresponding phase angle. Correlation tables for the other two otolith types revealed similar patterns, but of lesser magnitude.

TABLE 2. Fourier components from each of the three otolith types for five representative sampling sites. Amplitude (AMP), phase angle (PHA), and percent explained variance (EXV) by sampling site are presented for harmonics 1–10. Phase angles are presented in terms of the median value. N = nucleus as center for Fourier calculations; C = centroid as center for Fourier calculations; ASTL = left asteriscus; SAGL = left sagitta; LAPL = left lapillus.

			Harmonic 1				Harmonic 2				Harmonic 3				Harmonic 4				Harmonic 5			
			AMP		PHA		AMP		PHA		AMP		PHA		AMP		PHA		AMP		PHA	
	Site		Mean	SD	med.	EXV	Mean	SD	med.	EXV	Mean	SD	med.	EXV	Mean	SD	med.	EXV	Mean	SD	med.	EXV
ASTL	5	N	0.179	0.088	305.2	29.3	0.118	0.044	31.8	13.6	0.149	0.033	307.3	21.1	0.124	0.036	302.3	15.0	0.099	0.023	316.8	9.2
		C	0.050	0.021	166.2	2.8	0.170	0.044	329.2	33.2	0.164	0.039	302.5	30.6	0.112	0.032	319.9	14.9	0.088	0.028	326.7	9.3
	6	N	0.215	0.094	303.4	40.8	0.111	0.045	34.5	13.0	0.119	0.035	323.0	13.9	0.109	0.030	314.6	12.3	0.083	0.027	324.7	7.2
		C	0.038	0.015	161.2	2.1	0.157	0.048	25.5	36.0	0.124	0.040	318.2	22.7	0.098	0.030	323.1	15.4	0.088	0.030	34.8	11.5
	13	N	0.223	0.110	306.4	35.2	0.131	0.051	36.4	14.1	0.151	0.035	318.7	17.0	0.125	0.039	309.6	12.8	0.097	0.026	330.9	7.7
		C	0.051	0.019	159.8	2.6	0.192	0.052	25.9	36.7	0.160	0.040	311.2	26.0	0.111	0.031	325.8	13.4	0.103	0.029	49.0	11.0
	16	N	0.233	0.097	303.4	41.9	0.109	0.056	45.7	11.4	0.132	0.039	320.9	14.6	0.116	0.035	313.2	12.1	0.086	0.029	322.9	7.1
		C	0.039	0.020	163.4	2.0	0.164	0.057	33.6	34.7	0.133	0.046	318.5	23.9	0.098	0.034	329.9	14.1	0.092	0.037	37.9	11.4
	18	N	0.208	0.097	312.6	36.2	0.104	0.049	38.7	11.0	0.136	0.033	318.1	17.1	0.126	0.032	308.8	15.1	0.084	0.027	319.2	7.0
		C	0.044	0.017	168.7	2.4	0.159	0.049	325.2	31.8	0.153	0.038	313.0	29.6	0.103	0.029	322.8	14.3	0.089	0.028	42.0	10.1
SAGL	5	N	1.248	0.266	93.7	33.1	1.573	0.149	324.1	52.7	0.631	0.108	68.7	8.4	0.350	0.081	294.1	2.7	0.305	0.043	40.7	2.0
		C	0.079	0.023	290.4	0.2	1.731	0.130	343.2	89.5	0.145	0.046	112.2	0.7	0.477	0.062	334.8	6.8	0.164	0.038	62.9	0.9
	6	N	1.245	0.256	102.2	30.6	1.654	0.153	325.7	54.4	0.684	0.115	67.8	9.2	0.356	0.078	306.9	2.6	0.329	0.057	43.2	2.1
		C	0.088	0.026	261.5	0.2	1.794	0.151	343.9	88.9	0.142	0.058	77.4	0.7	0.503	0.073	342.0	7.0	0.189	0.044	53.0	1.0
	13	N	1.487	0.273	98.5	36.1	1.722	0.182	322.3	48.9	0.766	0.126	65.9	9.6	0.346	0.099	291.3	2.1	0.358	0.059	40.3	2.1
		C	0.094	0.026	281.2	0.3	1.909	0.175	343.7	88.9	0.178	0.057	94.0	0.9	0.519	0.083	338.8	6.6	0.217	0.043	60.1	1.2
	16	N	1.490	0.265	101.0	38.0	1.638	0.162	322.0	46.5	0.765	0.128	65.9	10.0	0.331	0.090	298.7	2.0	0.361	0.062	39.6	2.2
		C	0.086	0.030	277.4	0.2	1.825	0.161	342.6	88.4	0.175	0.058	94.8	0.9	0.511	0.078	343.3	6.9	0.204	0.049	58.9	1.2
	18	N	1.346	0.271	94.7	33.6	1.653	0.160	323.8	51.3	0.719	0.122	64.5	9.6	0.339	0.081	301.0	2.3	0.337	0.057	42.0	2.1
		C	0.094	0.030	270.6	0.3	1.829	0.163	344.1	88.8	0.157	0.065	89.0	0.8	0.516	0.070	340.3	7.1	0.186	0.047	60.5	1.0
LAPL	5	N	0.222	0.053	317.6	81.3	0.032	0.016	261.3	2.5	0.081	0.015	316.0	12.3	0.024	0.012	214.3	1.3	0.018	0.009	227.9	0.8
		C	0.003	0.002	104.5	0.1	0.048	0.018	332.9	26.0	0.064	0.015	287.1	44.4	0.036	0.012	61.9	15.6	0.021	0.011	289.3	5.4
	6	N	0.269	0.052	321.3	88.1	0.033	0.017	276.3	1.6	0.077	0.014	335.1	8.0	0.026	0.012	257.0	1.0	0.017	0.009	82.5	0.5
		C	0.003	0.002	86.8	0.2	0.046	0.018	34.9	27.2	0.058	0.015	305.2	43.3	0.037	0.011	91.8	18.8	0.019	0.009	299.3	5.4
	13	N	0.244	0.058	324.6	82.6	0.029	0.015	264.4	1.6	0.086	0.019	333.9	11.8	0.032	0.013	250.6	1.8	0.018	0.011	70.7	0.7
		C	0.004	0.002	63.7	0.2	0.042	0.019	46.9	18.4	0.075	0.017	305.5	54.3	0.036	0.011	110.0	13.7	0.026	0.009	322.7	7.0
	16	N	0.237	0.063	320.0	82.0	0.036	0.016	297.1	2.8	0.084	0.018	333.9	11.8	0.023	0.012	243.1	1.1	0.019	0.009	64.7	0.9
		C	0.004	0.002	103.4	0.1	0.056	0.022	331.6	31.6	0.063	0.016	306.1	40.0	0.039	0.012	84.1	16.4	0.023	0.009	307.0	6.1
	18	N	0.293	0.053	319.9	89.2	0.036	0.019	261.1	1.5	0.076	0.015	331.8	6.6	0.035	0.014	263.7	1.4	0.018	0.008	106.7	0.5
		C	0.004	0.002	54.7	0.2	0.040	0.018	259.9	19.7	0.065	0.015	290.8	49.7	0.039	0.011	84.5	19.6	0.020	0.008	302.2	5.1

TABLE 2. (*Concluded*)

			Harmonic 6				Harmonic 7				Harmonic 8				Harmonic 9				Harmonic 10			
			AMP		PHA		AMP		PHA		AMP		PHA		AMP		PHA		AMP		PHA	
	Site		Mean	SD	med.	EXV	Mean	SD	med.	EXV	Mean	SD	med.	EXV	Mean	SD	med.	EXV	Mean	SD	med.	EXV
ASTL	5	N	0.074	0.025	318.0	5.3	0.045	0.018	313.6	2.1	0.034	0.016	236.8	1.2	0.022	0.012	171.7	0.6	0.021	0.011	160.9	0.5
		C	0.047	0.021	167.1	3.0	0.032	0.015	77.0	1.4	0.025	0.015	187.2	0.9	0.021	0.013	209.3	0.7	0.020	0.010	242.3	0.6
	6	N	0.076	0.027	315.3	5.8	0.043	0.018	82.2	2.0	0.038	0.018	64.8	1.6	0.027	0.013	118.7	0.8	0.021	0.012	112.0	0.6
		C	0.046	0.023	50.0	3.8	0.039	0.021	74.3	2.5	0.025	0.013	218.8	1.2	0.021	0.013	197.2	0.9	0.022	0.011	250.0	0.9
	13	N	0.090	0.026	334.5	6.3	0.049	0.022	295.1	2.1	0.042	0.016	42.2	1.4	0.028	0.013	129.0	0.7	0.022	0.012	126.4	0.5
		C	0.049	0.021	53.2	3.0	0.038	0.016	71.8	1.8	0.028	0.014	256.6	1.0	0.022	0.012	257.0	0.6	0.025	0.015	259.4	0.9
	16	N	0.078	0.031	281.5	5.5	0.045	0.022	128.4	1.9	0.039	0.020	66.8	1.5	0.030	0.016	128.8	0.9	0.023	0.012	172.5	0.5
		C	0.047	0.025	71.4	3.5	0.039	0.021	90.4	2.4	0.031	0.017	248.6	1.6	0.025	0.014	255.1	1.1	0.027	0.014	283.8	1.2
	18	N	0.079	0.027	317.0	5.8	0.047	0.022	275.7	2.3	0.039	0.020	65.6	1.6	0.028	0.017	186.9	0.9	0.022	0.012	124.0	0.5
		C	0.048	0.025	83.8	3.5	0.034	0.020	98.1	1.8	0.030	0.016	234.1	1.5	0.022	0.014	243.5	0.8	0.026	0.013	255.7	1.0
SAGL	5	N	0.060	0.029	210.5	0.1	0.116	0.032	31.2	0.3	0.064	0.025	60.4	0.1	0.057	0.027	304.3	0.1	0.054	0.020	45.1	0.1
		C	0.123	0.034	331.6	0.5	0.126	0.029	47.2	0.5	0.044	0.019	285.1	0.1	0.087	0.020	38.0	0.2	0.031	0.015	246.0	0.0
	6	N	0.060	0.034	271.9	0.1	0.127	0.035	30.5	0.3	0.071	0.028	67.6	0.1	0.066	0.029	313.9	0.1	0.054	0.025	55.4	0.1
		C	0.150	0.041	337.9	0.7	0.141	0.033	42.4	0.6	0.060	0.027	317.4	0.1	0.084	0.027	45.5	0.2	0.035	0.018	296.3	0.0
	13	N	0.072	0.040	185.9	0.1	0.136	0.036	57.1	0.3	0.086	0.034	64.4	0.1	0.072	0.031	309.5	0.1	0.058	0.028	63.5	0.1
		C	0.135	0.040	325.7	0.5	0.154	0.034	50.6	0.6	0.057	0.030	304.3	0.1	0.093	0.028	46.1	0.2	0.044	0.023	280.2	0.1
	16	N	0.067	0.035	196.4	0.1	0.136	0.042	32.2	0.3	0.080	0.033	52.2	0.1	0.066	0.028	303.3	0.1	0.061	0.025	45.1	0.1
		C	0.147	0.048	335.2	0.6	0.147	0.038	40.7	0.6	0.059	0.026	300.0	0.1	0.093	0.026	40.4	0.2	0.039	0.019	290.0	0.1
	18	N	0.058	0.034	192.5	0.1	0.132	0.034	35.8	0.3	0.073	0.030	69.4	0.1	0.061	0.025	284.2	0.1	0.062	0.025	51.4	0.1
		C	0.144	0.040	329.6	0.6	0.144	0.033	50.7	0.6	0.049	0.025	295.9	0.1	0.088	0.027	47.1	0.2	0.036	0.018	268.2	0.0
LAPL	5	N	0.015	0.008	313.0	0.5	0.012	0.006	293.5	0.3	0.009	0.004	294.3	0.2	0.009	0.004	296.4	0.2	0.007	0.004	299.9	0.1
		C	0.012	0.006	306.0	2.1	0.011	0.006	309.2	1.6	0.011	0.005	294.9	1.4	0.008	0.004	287.5	0.9	0.006	0.003	299.2	0.5
	6	N	0.014	0.007	68.6	0.3	0.009	0.004	294.2	0.1	0.008	0.004	303.0	0.1	0.007	0.003	316.0	0.1	0.006	0.003	302.4	0.1
		C	0.010	0.005	236.5	1.6	0.008	0.004	300.0	0.9	0.007	0.004	301.3	0.9	0.005	0.003	67.2	0.3	0.004	0.002	103.4	0.3
	13	N	0.016	0.007	307.1	0.5	0.010	0.005	306.7	0.2	0.010	0.004	315.6	0.2	0.009	0.005	306.0	0.1	0.007	0.004	314.8	0.1
		C	0.012	0.006	208.3	1.8	0.010	0.005	282.0	1.3	0.010	0.005	298.3	1.2	0.005	0.003	230.0	0.4	0.006	0.003	127.0	0.4
	16	N	0.017	0.007	306.5	0.5	0.011	0.005	304.9	0.2	0.009	0.004	311.9	0.2	0.009	0.004	315.9	0.1	0.006	0.003	305.5	0.1
		C	0.012	0.005	314.0	1.7	0.010	0.005	291.1	1.2	0.009	0.004	305.4	1.0	0.006	0.004	83.6	0.4	0.004	0.003	64.6	0.3
	18	N	0.016	0.007	242.2	0.3	0.010	0.005	301.9	0.1	0.008	0.004	290.9	0.1	0.007	0.004	304.6	0.1	0.006	0.003	302.9	0.1
		C	0.010	0.005	274.6	1.4	0.008	0.004	308.3	0.9	0.008	0.004	295.7	1.0	0.006	0.003	163.9	0.5	0.005	0.002	274.7	0.3

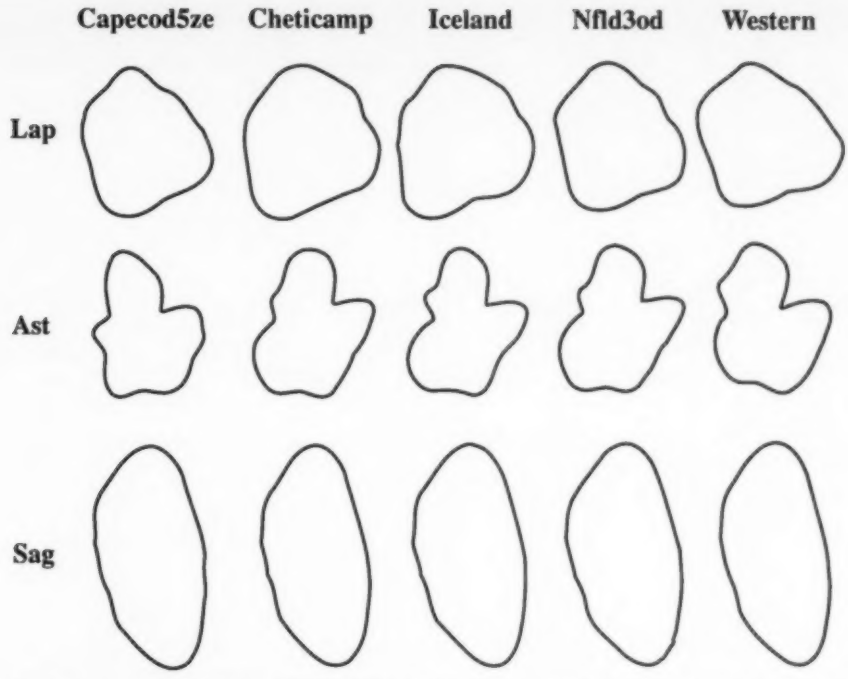

FIG. 6. Reconstruction of the mean otolith shape for five representative samples based on the mean amplitudes and median phases of the first 10 centroid-based harmonics. From top to bottom, the otolith outlines are those of lapilli (Lap), asterisci (Ast), and sagittae (Sag) for each of the five sample sites indicated. The relative sizes of the outlines are not drawn to scale.

With the exception of the phase angles, all of the shape variables were significantly correlated ($p < 0.01$) with both fish length and otolith length, which were in turn highly correlated with each other. Given the differences in length frequencies among samples, failure to remove this length effect could have introduced apparent (but unreal) shape differences among samples. A significant length effect ($p < 0.01$) was also evident when the amplitude variables were standardized to mean otolith radius, as is common practice. All standardized amplitudes were so affected. Since there was no advantage to using the standardized variables, we elected to remove the length effect from each of the unstandardized variables through an ANOCOVA (Table 3). Otolith length, rather than fish length, was treated as the covariate, since the otolith could be measured without significant measurement error and in the absence of distortion due to shrinkage or preservation.

Age was a significant modifier of several of the otolith shape variables. In light of the large disparity in age ranges between slow- and fast-growing samples, it was not possible to analyze all of the shape data for age effects in a single analysis. Accordingly, the data were arbitrarily subdivided into a slow-growing northern aggregation (Banq1, Banq2, Cheti, Gabarus, Green4Wd, Western, and Western88) and a fast-growing southern aggregation (Fundyrip, George5Zj, and Grandmanan). A two-way ANOVA (age and sample) of each of the shape variables for the left sagitta (centroid as centre), across ages common to all samples, resulted in relatively few significant age–sample interaction terms. Age was significant as a main effect for otolith area and the first six amplitudes in both the slow- and the fast-growing aggregations. In all cases, the estimated age parameters were internally conistent in that they formed a unidirectional trend. Neither the phase angle variables nor otolith perimeter varied significantly with age.

As a test of the strength of the age effect, the shape variables (with the length effect removed) from sites sampled in 1986 were entered into a discriminant analysis and used to predict the ages of the corresponding site sampled in 1988. For cod from Western Bank, ages 5–7 were predicted to within ± 1 yr with $\geq 80\%$ accuracy. The same accuracy was achieved for Browns Bank cod of ages 3–5. Classification accuracy was considerably less for older fish, but sample sizes were also low (<10) for those ages.

Sex effects on otolith shape were statistically significant, but of smaller magnitude than those of age. An analysis of age and sex by sample using two-way ANOVAs indicated that there were no significant sex effects (either as an age–sex interaction or a main effect) for amplitudes 1–4. On the other hand, otolith area, perimeter, amplitudes 5 and 7, and phase angles 1–5 all had a significant interaction and main effect in several samples. Few significant sex effects were observed in any of the fast-growing samples. Of all the variables, otolith area and perimeter appeared to have the strongest and most consistent response to sex.

There was evidence of sexually dimorphic growth in several of the sample sites (ANOVA of fish length by age and sex within samples), particularly at the slower growing sites (e.g., Banq2 and Cheti). Females tended to be larger at a given age than were males. However, the response was not common to all samples.

All of the samples had sex ratios not significantly different from unity, with the exception of Grandmanan (34 males, 65 females), Iceland (42 males, 18 females) and Western88 (97 males, 16 females).

TABLE 3. Common, within-group slope of otolith length removed as a covariate from each of the left-hand otolith shape variables. Where indicated (superscript "s") variables were square root transformed prior to ANOCOVA. Otolith perimeter (LNP) and area (LNSQA) were first ln transformed, as were their covariates. CFOCUS = distance from the nucleus to the centroid.

	Asteriscus		Lapillus		Sagitta	
Variable	Slope	SE	Slope	SE	Slope	SE
			Centroid as centre			
AMP 1	0.0568[s]	0.0034	0.0301[s]	0.0023	0.0197[s]	0.0008
AMP 2	0.1346	0.0034	0.0481	0.0026	0.1333	0.0016
AMP 3	0.0382	0.0035	0.0915	0.0037	0.0169	0.0011
AMP 4	0.0211	0.0027	0.0342	0.0015	0.0440	0.0011
AMP 5	0.0480	0.0024	0.0271	0.0012	0.0221	0.0008
AMP 6	0.0503[s]	0.0047	0.0383[s]	0.0038	0.0153	0.0007
AMP 7	0.0480[s]	0.0040	0.0557[s]	0.0033	0.0150	0.0006
AMP 8	0.0291[s]	0.0040	0.0103[s]	0.0033	0.0125[s]	0.0011
AMP 9	0.0275[s]	0.0037	0.0590[s]	0.0029	0.0085	0.0005
AMP 10	0.0374[s]	0.0034	0.0405[s]	0.0026	0.0116[s]	0.0009
LNP	0.8587	0.0113	0.8981	0.0077	1.0030	0.0095
LNSQA	0.6904	0.0090	0.8158	0.0075	0.8744	0.0084
			Nucleus as centre			
AMP 1	0.1226[s]	0.0088	0.1326	0.0076	0.0650	0.0053
AMP 2	0.1457[s]	0.0058	0.0468[s]	0.0070	0.1213	0.0020
AMP 3	0.0597	0.0028	0.0672	0.0018	0.0590	0.0021
AMP 4	0.0177	0.0029	0.0603[s]	0.0055	0.0284	0.0016
AMP 5	0.0346	0.0022	0.0892[s]	0.0044	0.0331	0.0009
AMP 6	0.0451	0.0022	0.0208	0.0009	0.0059	0.0007
AMP 7	0.0703[s]	0.0042	0.0402[s]	0.0036	0.0156	0.0006
AMP 8	0.0714[s]	0.0040	0.0493[s]	0.0032	0.0056	0.0006
AMP 9	0.0453[s]	0.0039	0.0416[s]	0.0031	0.0068	0.0005
AMP 10	0.0290[s]	0.0035	0.0295[s]	0.0029	—	—
LNP	0.8569	0.0114	0.8984	0.0077	1.0084	0.0095
LNSQA	0.6897	0.0090	0.8160	0.0075	0.8742	0.0084
CFOCUS	0.1208[s]	0.0074	0.1336	0.0077	0.0815	0.0052

Discriminant Analyses

The predictive power of the discriminant functions based on the otolith shape variables varied considerably with the otolith type, otolith centre, and shape variables which were used. All of the discriminant analyses of the otolith shape data were highly significant ($p < 0.001$). While the assumption of homogeneity of the variance–covariance matrices was not met, there was probably no substantive effect on the results, given the low F values, the conservative nature of the test used (Box's M), and the large sample sizes. This conclusion was confirmed by the classifications of the test samples, which reflected the patterns in classification evident in the known samples.

Comparisons of various discriminant analyses of the left sagitta demonstrated that otolith area and perimeter contributed most of the explanatory power to the analysis (Table 4). Analyses using only the amplitude variables were slightly less successful than were those which used only area and perimeter. Use of all variables, including the phase angle variables, provided the most accurate classification rates. There was no obvious difference in classification success between covariate-removed amplitudes and those standardized to mean radial length. Results obtained with either the centroid or the nucleus as the otolith centre were comparable. The sample from the Gulf of St. Lawrence was usually the most accurately classified individual sample (up to 58% in the test sample), while that from Iceland was poorly differentiated from the other samples (less than 20% accurate classification). In general, samples from the Gulf of Maine region were accurately classified as to region (up to 80% correct), but poorly differentiated within the region.

The similarity in classification success between discriminant analyses based on covariate-removed versus standardized amplitudes was somewhat surprising, given the fact that the covariate-removed amplitudes should have had all otolith/fish size effects removed while there was still a significant relationship between the standardized amplitudes and otolith/fish length. The similarity in classification success could have been a result of either different amplitude covariate slopes among the samples, or a weak relationship between the standardized amplitudes and otolith length. As a test of the former, discriminant analyses of the centroid-based sagittal shape variables were repeated using fish restricted to a length range of 50–60 cm. Classification success did not change in a consistent manner, confounding the interpretation. However, since a similar result was obtained when the same analysis was conducted with the standardized amplitudes, the length effect may be relatively minor. Whatever length effect was present was probably incompletely removed by both the ANOCOVA and the standardized variable methods.

Discriminant functions based on 1986 collections classified both 1986 and 1988 samples as to geographic origin with similar levels of accuracy. In the case of the centroid-based sagitta variables, 6% of the 1988 sample from Browns Bank was cor-

TABLE 4. Summary of discriminant analyses of the left sagitta comparing the effects of choice of otolith centre (nucleus versus centroid), "standardized" (AMPs divided by mean radial length) versus covariate-removed data, and the various otolith size and shape variables. Classification success refers to classification of samples not used in the discriminant analysis. The discriminant functions were calculated on the basis of the individual samples, not those aggregated into regions.

	Classification success (%)					
		By region[a]				
Analysis type	By sample	Gulf of Maine	Eastern Scotian Shelf	Gulf of St. Lawrence	Newfoundland	Iceland
Centroid; LNP, LNSQA only	17.6	61	37	58	58	7
Centroid; AMPs only	15.0	61	28	35	47	10
Centroid; no PHA variables	18.3	70	45	40	54	10
Centroid; all variables	18.6	77	45	47	58	17
Nucleus; no PHA variables	21.4	73	48	39	49	13
Nucleus; all variables	23.0	80	45	42	57	17
Centroid; no PHA, standardized AMPs	21.2	69	46	54	58	14
Centroid; standardized AMPs only	13.0	61	28	19	43	3

[a]Samples included in each region: Gulf of Maine = samples 3, 4, 5, 7, 9, 10, 11, 14, 17; Eastern Scotian Shelf = samples 1, 2, 8, 12, 18, 19; Gulf of St. Lawrence = sample 6; Newfoundland = samples 15, 16; Iceland = sample 13.

TABLE 5. Summary of discriminant analyses of the left otolith comparing the predictive power of otolith type (AST = asteriscus; LAP = lapillus; SAG = sagitta), choice of otolith centre (nucleus versus centroid), and "standardized" (AMPs divided by mean radial length) versus covariate-removed data. All discriminant analyses used the Fourier amplitudes (AMPs), LNP (perimeter), and LNSQA (area), without the PHA variables. Classification success refers to classification of samples not used in the discriminant analysis. The discriminant functions were calculated on the basis of the individual samples, not those aggregated into regions.

	Classification success (%)					
		By region[a]				
Analysis type	By sample	Gulf of Maine	Eastern Scotian Shelf	Gulf of St. Lawrence	Newfoundland	Iceland
AST centroid	12.6	69	28	42	25	14
AST centroid; standardized AMPs	12.4	67	37	49	14	6
AST nucleus	10.9	73	31	18	22	4
AST nucleus; standardized AMPs	11.5	72	33	32	16	4
LAP centroid	11.4	76	41	3	25	12
LAP centroid; standardized AMPs	15.1	75	37	15	37	12
LAP nucleus	13.9	71	33	6	40	13
LAP nucleus; standardized AMPs	14.4	69	35	12	32	22
SAG centroid	18.3	70	45	40	54	10
SAG centroid; standardized AMPs	21.2	69	46	54	58	14
SAG nucleus	21.4	73	48	39	49	13
SAG nucleus; standardized AMPs	23.0	75	49	33	52	13

[a]Samples included in each region: Gulf of Maine = samples 3, 4, 5, 7, 9, 10, 11, 14, 17; Eastern Scotian Shelf = samples 1, 2, 8, 12, 18, 19; Gulf of St. Lawrence = sample 6; Newfoundland = samples 15, 16; Iceland = sample 13.

rectly identified as being from Browns Bank, as compared with 13% of the 1986 test sample. However, 67% of the 1988 sample was correctly assigned to the Gulf of Maine region (versus 48% of the 1986 sample). In the case of the Western Bank sample, 7% of the 1988 sample and 17% of the test 1986 sample were correctly classified. However, 48% of the 1988 sample and 45% of the test 1986 sample were assigned correctly to the eastern Scotian Shelf. For reasons discussed later, neither the Browns Bank nor the Western Bank samples were particularly accurately classified. However, misclassification errors were similar across sampling years.

Comparison of discriminant analyses among the three otolith types indicated that the sagitta tended to give the most accurate classifications (Table 5). There were no consistent differences between the two otolith centres (nucleus versus centroid) within a given otolith type, nor between covariate-removed and standardized amplitudes. On the other hand, there were consistent differences in classification rates among the otolith types. For instance, lapillar shape was a much better indicator of Icelandic origin than was asteriscal shape, while the converse was true for the Gulf of St. Lawrence. All otolith types performed well in identifying cod from the Gulf of Maine.

The results of the discriminant analysis of the left sagitta indicated that otolith area, perimeter, and the lower order amplitudes were among the most influential variables in the analysis (Table 6). While the first six discriminant functions were all

TABLE 6. Statistics and unstandardized function coefficients from the discriminant analysis of the centroid-based, left sagitta shape variables based on covariate-removed data. AMP = Fourier amplitude; LNP = ln-transformed otolith perimeter; LNSQA = ln-transformed otolith area; superscript "s" = square root transformed.

	Discriminant function						
Shape variable	1	2	3	4	5	6	7
AMP1^s	−2.688	0.710	15.991	−15.181	−32.433	8.742	20.279
AMP2	3.568	7.783	4.859	8.005	9.575	−0.926	6.001
AMP3	3.491	3.331	−0.417	13.368	12.742	−3.076	−19.312
AMP5	−8.089	−0.387	−10.678	14.149	16.687	15.746	0.732
AMP6	−5.399	−1.664	−4.423	33.392	−7.760	−5.594	17.828
AMP8^s	−4.065	4.285	−3.987	−2.536	12.025	4.426	5.734
AMP9	14.395	−6.152	19.240	10.861	12.468	−20.054	−6.841
LNP	33.457	−9.712	−15.573	8.984	−0.097	10.914	3.549
LNSQA	−27.624	−8.365	34.693	28.839	12.859	−11.604	52.445
Constant	−38.753	9.849	22.176	5.237	7.681	−12.844	7.241
Eigenvalue	0.929	0.319	0.095	0.057	0.027	0.022	0.018
Percent of variance	62.47	21.45	6.43	3.88	1.83	1.53	1.23
Cumulative percent	62.47	83.91	90.34	94.22	96 05	97.58	98.81
Significance	0.000	0.000	0.000	0.000	0.016	0.069	0.209

statistically significant, the first two functions explained almost 84% of the variance.

To determine if the probability of classification increased with proximity to the source sample, a discriminant analysis of the centroid-based sagitta shape data was carried out in which the discriminant functions were based on the aggregated samples listed in the footnote to Table 4. The rates of incorrect classification were then plotted against the distances to the other regions (Fig. 7). No relationship was evident, suggesting that regions were not simply misclassified to the nearest alternative region. However, if the Iceland sample was excluded from the analysis, misclassification rate was significantly and inversely correlated with the distance to the misclassified region.

Classification success increased markedly as the number of groups entered into the discriminant analysis was reduced. While this is a phenomenon common to most discriminant analyses, it appeared to be particularly marked in this study. For instance, analysis of three widely spaced samples (Fundyrip, Cheticamp, and Nfld3Ld) resulted in an overall classification success of 78% for the test sample. There was greater than 89% differentiation between the Cheticamp and Fundyrip samples. Conversely, analysis of the seven Gulf of Maine samples resulted in 30% classification accuracy by sample, which is not much greater than the overall classification success with all 19 samples. Therefore, sample number by itself did not explain apparently high misclassification rates among some samples.

Since the classification success of a given sample varied with the otolith type that was used (Table 5), and since no one otolith type provided the best classifications for all samples, use of all three otolith types could provide more information about sample origin than any one otolith type alone. Accordingly, the centroid-based shape variables (without phase angles) from each of the three left-hand otoliths of each fish were treated as independent variables in a discriminant analysis. Overall classification success of the test sample (25.4%) was higher than that of any individual otolith type (Table 5) and appeared to accurately identify regional aggregations of samples (Fig. 8). While not used to construct the discriminant functions, the 1988 samples were also reasonably well classified on the basis of the corresponding 1986 samples. Of the 24 shape variables that entered the discriminant analysis, only four consisted of higher order harmonics (e.g., harmonics >6). Otolith area and perimeter from all of the otolith types were influential variables; sagittal and lapillar area and perimeter made up four of the first seven variables entered into the analysis. Overall, the shape variables derived from sagittae were more influential than the shape variables from the other otolith types. As was the case with the analysis of the sagittae alone, both the Fourier amplitudes and otolith area/perimeter variables by themselves were moderately successful in classifying samples, although considerably less so than was the case with all non-phase-angle variables combined.

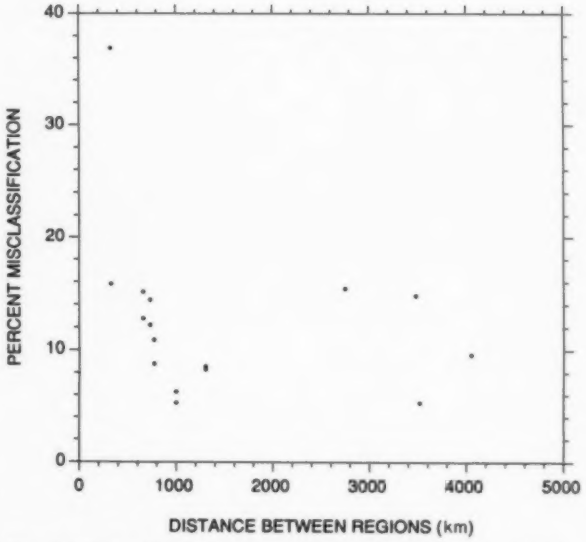

FIG. 7. Misclassification by region as a function of the distance to the approximate centre of the samples comprising that region. Samples included in each region are identified in the footnote to Table 4.

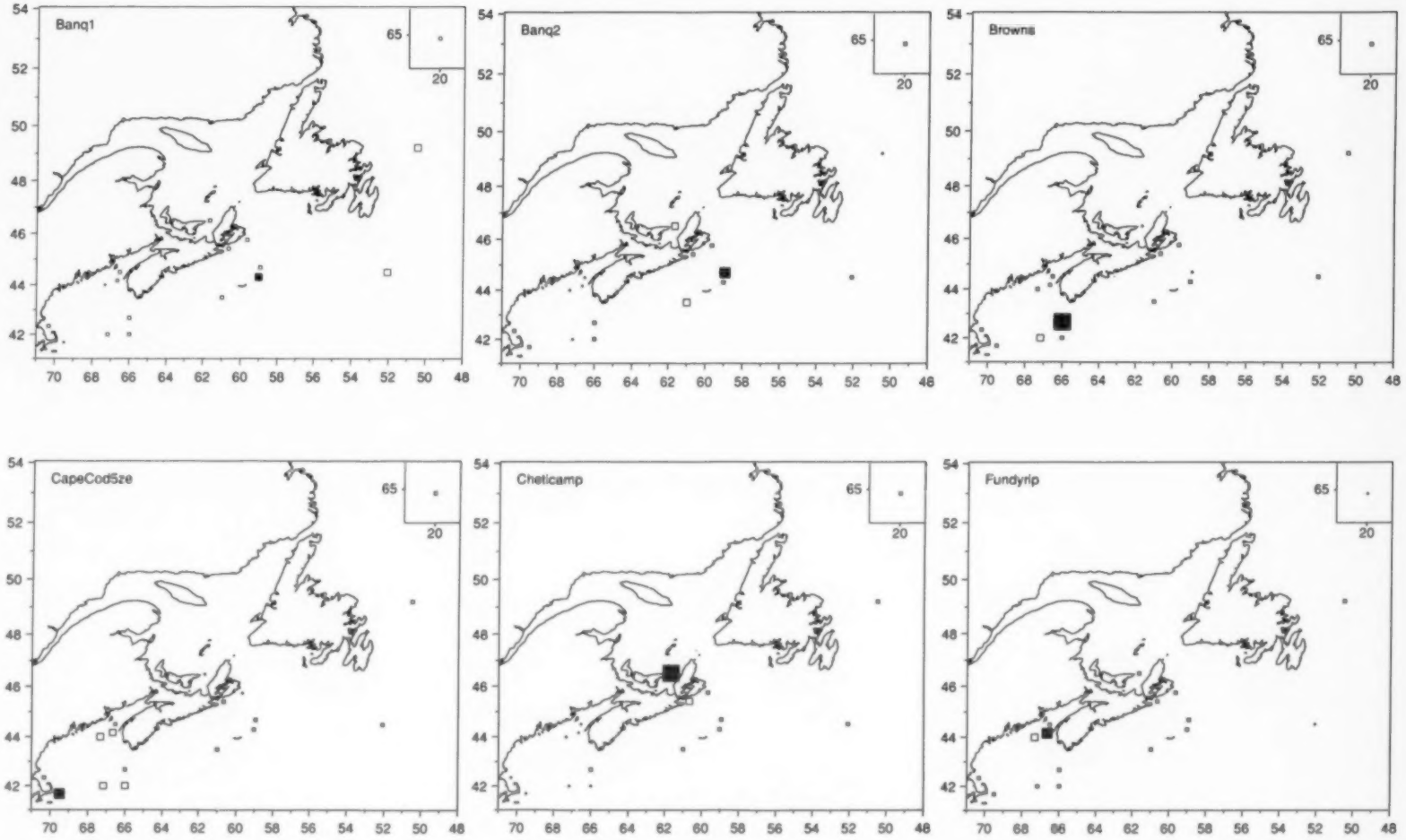

FIG. 8. Expanding symbol plots of percent classification success based on the discriminant analysis of the 1986 samples using all three otolith types (centroid as origin). The actual sample collection site is shown as a solid square. The sample from Iceland is shown as an insert in the top right-hand corner. The scale for the expanding symbols is presented to the right of the last panel. (*Fig. 8 continued next page*)

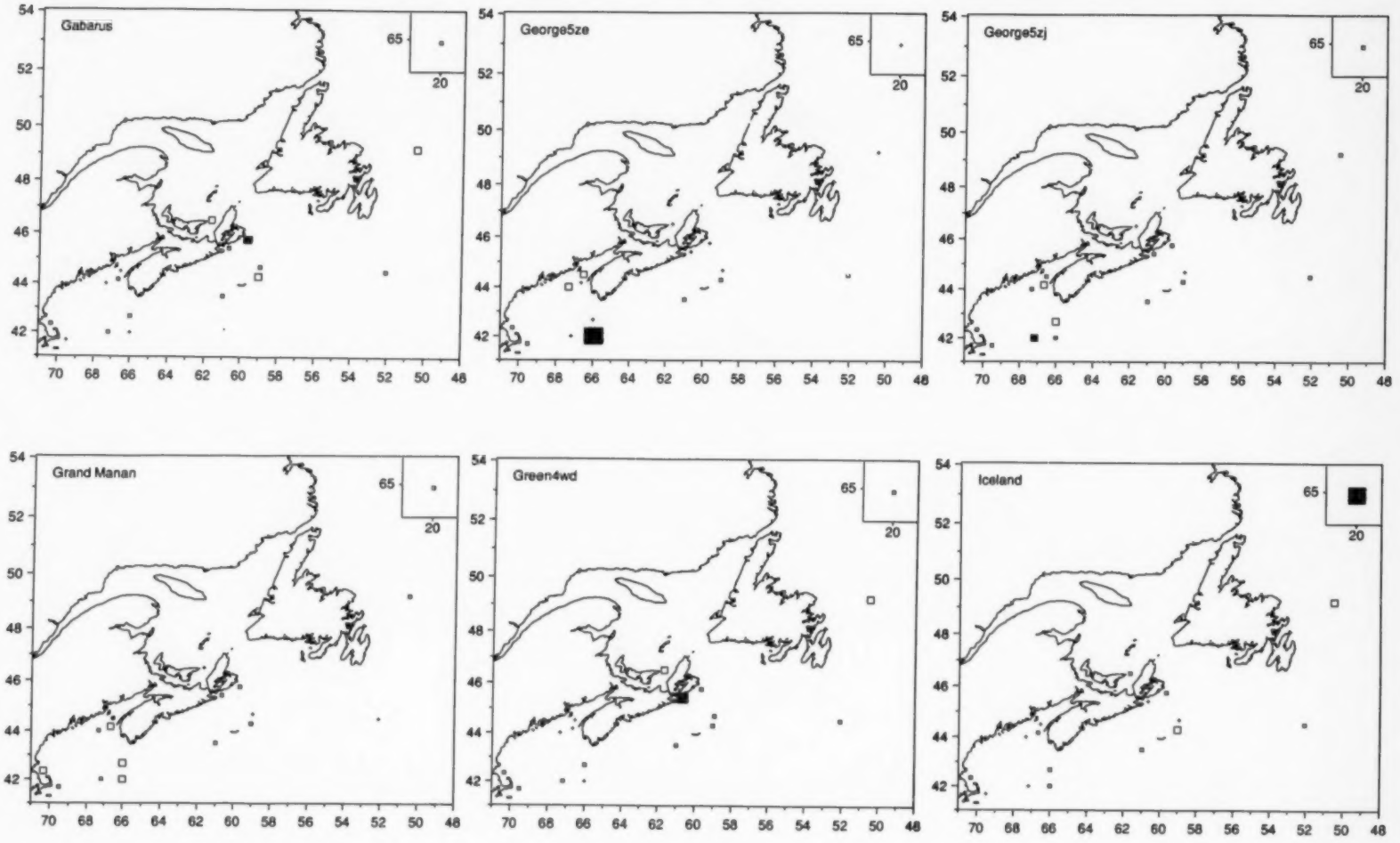

FIG. 8. (*Continued next page*)

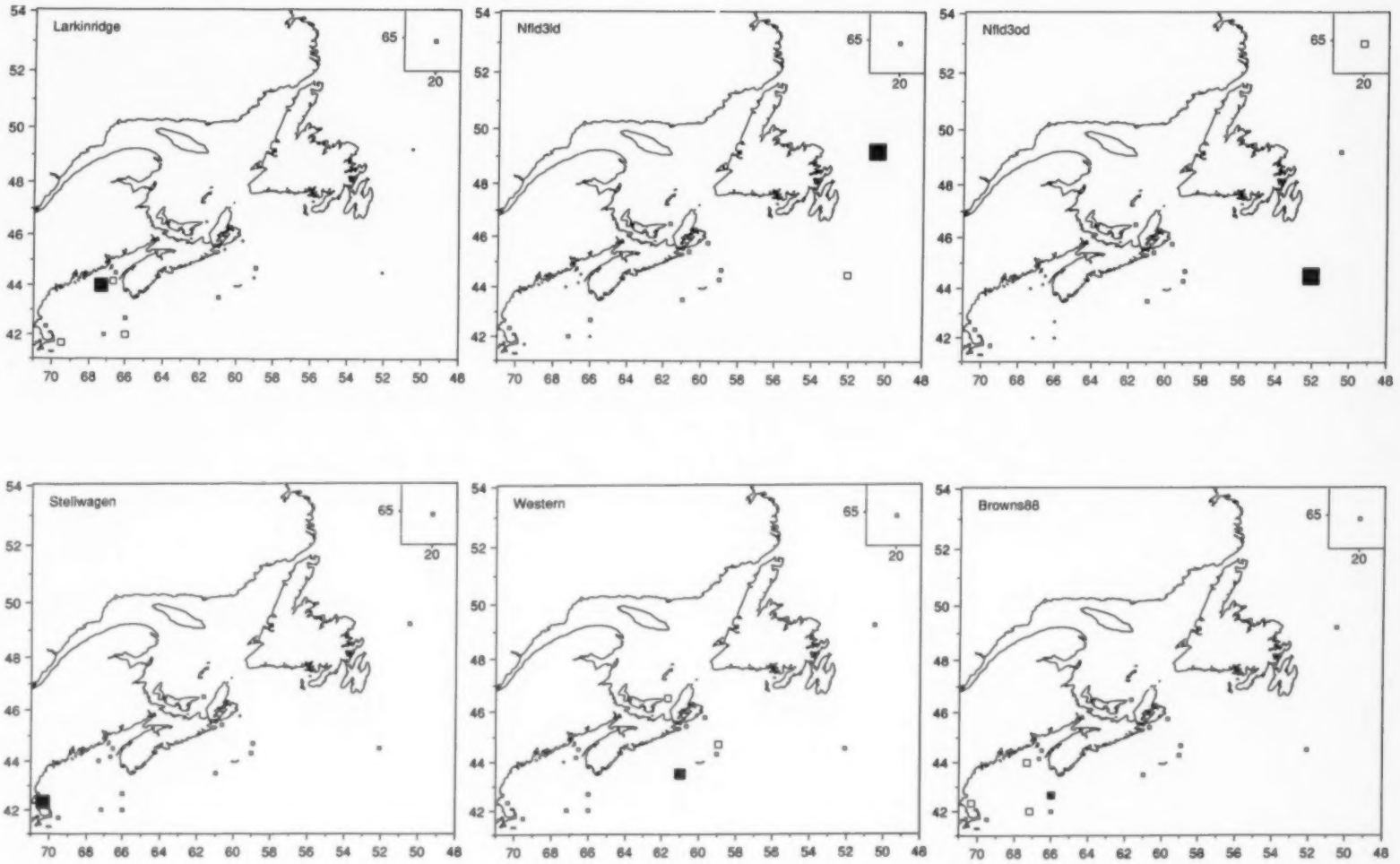

FIG. 8. (*Concluded next page*)

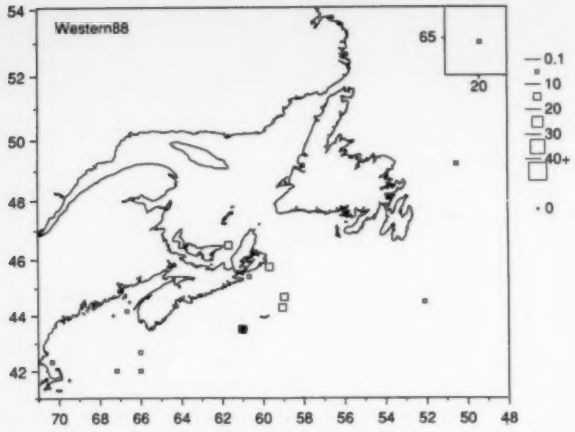

FIG. 8. (*Concluded*)

Otolith Shape as a Function of Growth Rate

Examination of Fig. 8 indicates that, in general, samples from a given region were classified into the same region. However, misclassification rates among certain samples did not follow this pattern. For instance, samples from Newfoundland (Nfld3ld and Nfld3od) were often misclassified onto the eastern Scotian Shelf (Banq1, Banq2, Gabarus, Green4WD, Western, and Western88) and vice versa. As will be discussed later, large-scale dispersion between these regions appears unlikely, but cannot be ruled out. However, classification errors between Iceland and the other regions cannot realistically be assigned to fish movements. Reexamination of Fig. 8 indicates that all samples were most often misclassified to samples with similar growth rates. This is most evident in the Iceland sample, which has growth rates that are comparable with those of the eastern Scotian Shelf and Newfoundland.

Classifications mistakenly assigned to samples of similar growth rates suggest that otolith shape is correlated with growth rate. To test this hypothesis, the discriminant function values (from the combined-otolith type, centroid-based discriminant analysis) corresponding to each of the sample means (sample centroids) were regressed against mean annual somatic growth rate (Fig. 9). There was a significant relationship between growth rate and the values of the first discriminant function corresponding to the sample centroids ($p < 0.01$, $R^2 = 0.78$), indicating that growth and otolith shape were highly correlated. The residuals were curvilinear, suggesting that the relationship was not strictly linear. However, the second discriminant function was not significantly correlated with the residuals. Both somatic growth rate and otolith growth rate were related to the first discriminant function in a similar manner, indicating that otolith shape was related to growth rate in general.

The relationship between growth rate and otolith shape was both persistent and independent of variable type. Significant regressions between growth rate and the first discriminant function values at the sample centroids were observed when each of the otolith types was analyzed separately. The relationship was also significant if only the amplitudes were entered into the discriminant analysis. Similar results were obtained with or without the phase angle variables, if only otolith area/perimeter were analyzed, or using only the standardized amplitudes. ANOCOVA indicated that growth rate was a significant covariate of most (but not all) shape variables.

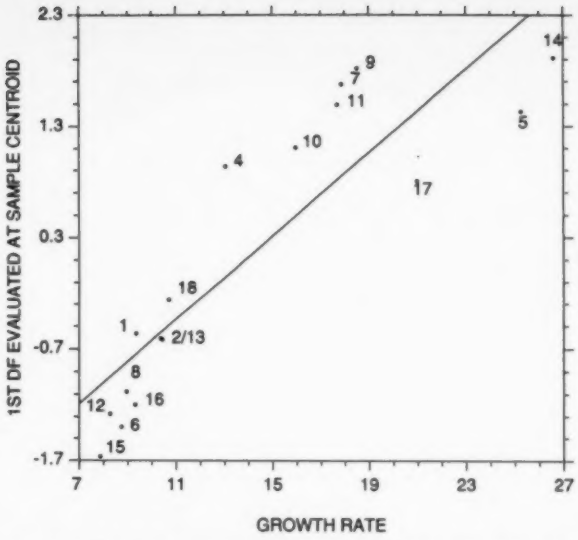

FIG. 9. Relationship between mean annual growth rate (cm/yr) of the fish and otolith shape (as indicated by the value of the first discriminant function for all three centroid-based otolith shapes, evaluated at each sample centroid). Sample numbers are shown on the plot. The regression line is described by $Y = -2.493 + 0.187X$ ($R^2 = 0.78$).

Given the strong correlation between growth rate and the first discriminant function, and in light of the substantial contribution of the first discriminant function to explained variance (~65%), it is possible that much of the observed classification success of the various discriminant analyses was due to environmentally induced differences in growth rate among samples, rather than genetic differences in otolith shape. In order to test this hypothesis, the sagittal shape data were reanalyzed after otolith growth rate was removed as a covariate. Classification success declined substantially, although some test samples were still classified with 30–40% success. Similar results were obtained if otolith area was removed as a covariate from the shape variables. However, the sample which would be expected to have the greatest genetic differentiation from the other samples (Iceland) was still classified poorly (0–20% success). Accordingly, the genetic component of the observed differences in otolith shape did not appear to be as strong as that of the environmental component.

A further test for genetic differences in sagittal shape among samples was made by analyzing five samples (Banq1, Gabarus, Iceland, Nfld3od, and Western88), carefully matched for growth rate so as to control for the growth effect. Only ages 6 and 7, subsampled to produce the same age ratio in each sample, were analyzed so as to control for any age effect. The resulting classifications of the test sample were usually highest for the correct area, but were not particularly high (20–50%) given the low numbers of samples in the analysis. The Iceland sample was classified correctly in only 20% of the cases. As a result, the environmental component of otolith shape would appear to be considerably stronger than the genetic component, but the latter may still have contributed to the significantly better than random classifications of the test sample.

A final test of the influence of growth rate on otolith shape was made by comparing the classification of the 1988 samples (which were not used in the preparation of the discriminant

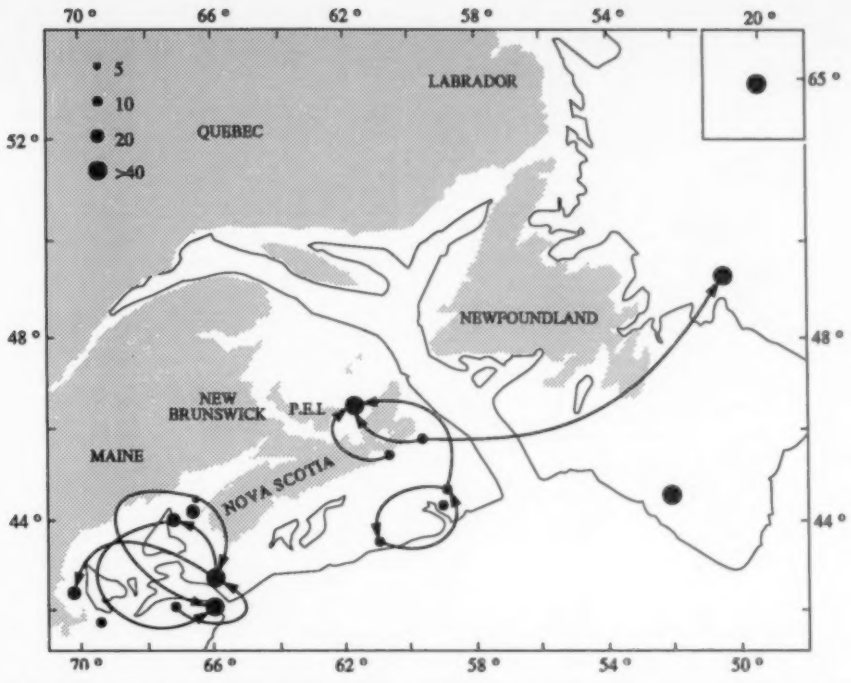

FIG. 10. Summary map depicting the classification patterns resulting from the centroid-based discriminant analysis of all three left-hand otolith types combined. The expanding symbols represent the percentage of fish entered into the discriminant functions which were correctly classified. Vectors connecting samples represent misclassification errors which exceeded 15%. None of the samples were mistakenly assigned to a single sample at a rate of more than 20%. Misclassification errors of less than 15% are not shown. The 200-m contour is shown. The sample from Iceland is shown as an insert in the top right-hand corner.

functions) with that predicted on the basis of the growth rates. As predicted, the 1988 Browns Bank sample, which grew at about the same rate as the 1986 sample, was classified similarly in both samples. The 1988 Western Bank sample, which grew more slowly than its 1986 counterpart, was classified into other groups with more similar growth rates. These results suggest that the utility of otolith shape for stock identification may lie with relative year-to-year constancy in the growth rate of a given stock.

Geographic Patterns in Otolith Shape

There were consistent similarities among the classification patterns from each of the otolith types, whether based on the nucleus or centroid as the otolith centre. As summarized in Fig. 10, samples from within broad geographic regions, such as the Gulf of Maine, were well differentiated from other regions, but poorly discriminated from samples within the same region. Otoliths from Gulf of St. Lawrence (Cheticamp) cod tended to be accurately classified (40–60% accuracy), but classification errors to the northeastern Scotian Shelf were common. Classification errors in one of the Newfoundland samples were most often linked to the other sample from the same area (see also Fig. 8). The Iceland sample was the anomaly here; misclassifications from the Iceland sample were often directed to the Scotian Shelf, rather than the geographically adjacent Newfoundland samples.

Discussion

Can otolith shape be used as a tool for differentiating among cod stocks? The answer would appear to be "sometimes." Under Ihssen et al.'s (1981) definition of a stock as an "intraspecific group of randomly mating individuals with temporal or spatial integrity," stocks are definable using population characteristics such as growth rate, among other things. The results of our study indicate that cod from stocks with clearly different growth rates can be reasonably well differentiated on the basis of otolith shape alone. Both genetic and environmental influences undoubtedly contributed to the observed stock differences in otolith shape; however, the latter appeared to be more influential. With the influence of the environment being paramount, the utility of otolith shape for stock identification would depend on the relative constancy of the environment in a given stock area, integrated over the lifetime of the fish. For all but short-lived species, this would be a reasonable assumption, since year-to-year differences in the environment would be smoothed out over the lifetime of the fish.

Those cod samples which could be classified into discrete aggegations using otolith shape analysis largely conformed to stock boundaries determined with other techniques. Tagging (McKenzie 1956; Templeman 1962; Wise 1963), meristics and morphometrics (Templeman 1962; Lear and Wells 1984), ichthyoplankton surveys (O'Boyle et al. 1984; Hurley and Campana 1989), and parasite loads (Scott and Martin 1957) are

all consistent with the view that cod from around Iceland, Newfoundland, the southern Gulf of St. Lawrence, the Scotian Shelf, and the Gulf of Maine are largely discrete aggregations. Within each of these aggregations, extensive mixing is believed to occur, resulting in complex stock mixtures. This is particularly true of the Gulf of Maine, where tagging studies have demonstrated extensive mixing throughout the Gulf, and at the entrance to the Gulf of St. Lawrence, where large-scale seasonal migrations from the Gulf to the Scotian Shelf have been shown to occur (Wise 1963; Halliday 1973; W.T. Stobo, Bedford Institute of Oceanography, Dartmouth, N.S. B2Y 4A2, personal communication). With the exception of the Iceland sample, otolith shape analysis distinguished among the above five regions with reasonable accuracy. However, sites within the Gulf of Maine could not be clearly differentiated, nor could those in and around the entrance to the Gulf of St. Lawrence. As a result, we could not determine whether our within-region results were due to the innate imprecision of otolith shape analysis or to true mixing within the region. Similarly, protein electrophoresis (Cross and Payne 1978; Mork et al. 1985) and mitochondrial DNA studies (Smith et al. 1989; Carr and Marshall 1991; Dahle 1991) have been largely unsuccessful in distinguishing among cod populations.

The finding that otolith shape changed largely in response to differences in growth rate was unexpected. Numerous studies have documented growth-related changes in the size of the otolith relative to that of the fish (Templeman and Squires 1956; Boehlert 1985; Mosegaard et al. 1988; Secor and Dean 1989; Campana 1990; Casselman 1990). In the above studies, slow-growing fish formed larger otoliths than did fast-growing fish of the same size. However, the changes in relative size noted in the above studies were never associated with changes in shape, nor was there reason to expect them to be; a change in size need not induce any change in shape. The apparently common response of relative otolith size to a change in growth, especially growth changes induced by environmental conditions, indicates that the otolith growth process is highly susceptible to environmental effects. While we have no experimental evidence that otolith shape changed in response to environmental versus genetic growth differences, the former seems more likely. The large-scale gradients in cod size-at-age observed in this study are, at least superficially, correlated with similar gradients in water temperature (Drinkwater and Trites 1987). Temperature is known to be a primary modifier of growth rate in fish (Brett 1979). A growth effect on otolith shape is also evident with respect to the age, sex, and year-class differences observed in this study; in each instance, the presence of significant otolith shape differences among groups could be linked to differences in growth rate.

The finding that otolith shape was highly correlated with growth rate would appear to explain most, if not all, of the significant otolith shape differences observed within other species. Otolith shape accurately discriminated among a number of lake whitefish (*Coregonus clupeaformis*) stocks in Lake Huron (Casselman et al. 1981). However, subsequent reexamination of the data revealed that stocks were discriminated only where mean stock growth rates were substantially different and failed to be differentiated when growth rates were similar. Growth rate effects also appeared to account for the observed differences among age groups. In a study of herring otoliths, Bird et al. (1986) reported relatively few significant differences among the first 10 harmonics when comparing between Alaskan stocks (*Clupea pallasi*) or between widely spaced Atlantic stocks (*Clupea harengus*) (Georges Bank and Gulf of St. Lawrence herring). Conversely, most of the lower order harmonics differed significantly in a comparison of the Alaskan stocks with the Gulf of St. Lawrence stock, and even more so with the Georges Bank stock. This pattern of similarities and differences accurately reflected corresponding differences in growth rates. While growth rates were not reported in Bird et al.'s (1986) paper, other sources indicate that the two Alaskan stocks had similar lengths at age 5 (245–255 mm) (Lebida 1987), while the Georges Bank and Gulf of St. Lawrence herring were considerably larger (280–310 mm) (Winters 1976; Anthony and Waring 1980). The size-at-age discrepancy was largest between the Alaskan fish and the Georges Bank fish. Since there is no reason to expect the Georges Bank – Alaskan herring distinction to be any greater than the Gulf of St. Lawrence – Alaskan distinction, growth-related otolith shapes appear to better account for Bird et al.'s (1986) results than do stock-specific differences. Smith (1992) came to a similar conclusion in a recently published study on Pacific deep slope red snapper (*Etelis carbunculus*).

A final test of the hypothesis linking otolith shape with growth rate was available in Castonguay et al.'s (1991) detailed study of Atlantic mackerel (*Scomber scombrus*) otolith shapes. Castonguay et al. (1991) reported relatively few significant differences in lower order harmonics between northern and southern contingents on the Atlantic coast, but numerous differences between either contingent and the North Sea stock. Classification of test samples reflected these differences, in that discrimination between northern and southern fish was relatively poor (36–68%), but discrimination between either northwest Atlantic contingent and the North Sea stock was much better (60–91%). Comparison of growth rates among the samples indicated relative similarity between the northern and southern contingent (e.g., 29.1 cm at age 3 for the southern contingent; 29.5 cm at age 3 for the northern contingent) (Anderson and Paciorkowski 1980) and greater differences with the North Sea stock (32.9 cm at age 3) (Hamre 1980). Therefore, either stock effects or growth rate differences could be used to explain the otolith shape differences between the North Sea and the northwest Atlantic stocks. However, Castonguay et al.'s (1991) greatest discrimination success (87–91%) occurred in a comparison of southern contingent otoliths with those from the North Sea. There is little genetic basis for expecting southern contingent mackerel to be any more distinct from North Sea mackerel than are northern contingent fish. However, growth rate differences are maximal between the southern contingent and North Sea fish; therefore, on the basis of the growth rate hypothesis, one would expect the best discrimination between these two groups of fish. Castonguay et al.'s (1991) observations of numerous otolith shape differences among age groups and year-classes are also consistent with mackerel's propensity for changes in growth rate with year-class strength (Anderson and Paciorkowski 1980; Hamre 1980).

The conclusion that the observed otolith shape differences were more a function of the environment than genetics is largely based on the analysis of the Icelandic cod samples. There is no evidence of mixing of Icelandic cod with those from the eastern coast of Canada (Templeman 1962), indicating that the former should be genetically distinct, at least relative to the other samples. However, the Icelandic cod sample tended to be among the least accurately discriminated by otolith shape analysis. Since the growth rate of the Icelandic cod was very similar to that of the Scotian Shelf cod, such would appear to explain the

consistent misclassification of Icelandic cod as Scotian Shelf cod. It would also point to an environmental rather than a genetic basis for the growth rate effect on otolith shape. However, in the context of a stock identification study, the environmental effect on otolith shape is probably no more severe than that on any other stock discrimination technique. Both meristics and morphometrics, the traditional mainstays of stock identification, are now known to be heavily influenced by environmental (particularly temperature) effects (Ihssen et al. 1981; Blouw et al. 1988; Swain et al. 1991). Population characteristics are also so affected. As long as the environmental effect is recognized, and as long as year-to-year shifts in environmental conditions can be incorporated into the conclusions, stock identification using nongenetic protocols should not be compromised. Indeed, after a comparison of a broad suite of morphometric, meristic, and genetic stock indices of lake whitefish, Casselman et al. (1981) concluded that characters influenced by environmental differences could be just as reliable in indicating stock discreteness as genetically based characters.

Given that otolith shape analysis has some utility as a stock identification tool, what is the best way to go about it? In terms of the otolith type selected for use, the sagittae would appear to be as good or better than the other otolith types. Sagittae are routinely used for age determination, and because of their greater size, are easiest to collect from cod (and most other species). In addition, in this study, asteriscal shape tended to be most variable, both within and among fish, while the lapilli tended to be relatively featureless. Both of these latter otolith types resulted in weaker group discrimination than did the sagittae. These results are contrary to Radtke's (1984) observation of noticeable lapillar shape differences between inshore and offshore cod.

Selection of the otolith centre used in the Fourier analysis had little impact on the eventual classification success. Biologically, one would expect the otolith nucleus to be the most useful reference point, given its status as the origin of growth of the otolith. However, use of the biological centre did not result in any clear advantage over the mathematical centroid, and indeed, required operator intervention. In any case, the lapillar nucleus was almost impossible to view externally. There are also mathematical reasons for selecting the centroid for use in the Fourier analysis; use of the sometimes eccentrically located nucleus can potentially result in "centering error" (Full and Ehrlich 1982), although the latter does not apply to analysis of shapes which are unravelled from homologous landmarks (such as the postrostrum). For this reason, shapes should be unravelled from the same landmark on all otoliths. The presence of homologous landmarks also makes the Fourier analysis of otoliths more rigorous than that of many other objects (Younker and Ehrlich 1977; Full and Ehrlich 1982), since it reduces the variability of the Fourier amplitudes and makes the Fourier phase angles interpretable. Unfortunately, there is no straightforward way to normalize the phase angles prior to incorporation into the multivariate analysis. Most, if not all, workers have excluded Fourier phase angles from their studies (Younker and Ehrlich 1977; O'Higgins and Williams 1987).

One of the more surprising findings of this study concerned the removal of fish size effects from the otolith shape variables. Most commercial image analysis systems which incorporate Fourier analysis compute the standardized Fourier amplitudes (amplitude divided by the mean radial length). This study demonstrated that the standardized amplitudes do not completely remove the effect of fish length. However, the more statistically rigorous procedure of removing either fish or otolith length as a common, within-group covariate (Reist 1985; Claytor and MacCrimmon 1987) also failed to remove all of the length effect. Neither approach seemed to be clearly superior to the other. Since the standardized amplitudes can be calculated automatically, they are logistically easier to use. Given the widespread variation of the otolith – fish length relationship with growth rate (Templeman and Squires 1956; Boehlert 1985; Mosegaard et al. 1988; Secor and Dean 1989; Campana 1990; Casselman 1990), and given that such an effect could confound the variation in otolith shape, the Fourier amplitudes are best standardized using otolith length rather than fish length, irrespective of the standardization approach used. Note also that otolith shape varies ontogenetically independent of growth rate (Harkonen 1986), such that otoliths from young fish become increasingly difficult to differentiate among species as size decreases. Accordingly, otolith shape analysis may be of negligible value to stock identification if applied to sexually immature fish.

There is no reason to expect the principles of otolith shape analysis discussed above to differ among species; otolith growth processes appear to be ubiquitous to all species (Campana and Neilson 1985). However, the specifics may well change. Otolith shape differs substantially among species (Nolf 1985; Harkonen 1986), suggesting that the most influential shape variables may well change across species. Even the preferred otolith type may differ among species, since the sagitta is the largest otolith in many, but not all, teleosts (e.g., Cypriniformes). However, it appears unlikely that selection of the largest otolith type, whichever it is, would prove to be a bad decision.

While otolith shape analysis appears to be a useful stock identification tool in many situations, its use would be inappropriate under some conditions. Obviously, otolith shape will not differentiate well among populations with similar growth rates. It is also not suited to the identification of individual fish, since slow-growing fish from the fast-growing stock will be classified with the slow-growing stock and vice versa. Finally, shape analysis (and most other stock identification techniques) is not suited for addressing the site affinity problem; that is, the technique does not have sufficient power to determine whether individual fish are apparently misclassified because of the imprecision of the methodology or because the fish is actually a stray from another area. Otolith shape will also not indicate where a fish was hatched, although it will point to the area where it grew up.

The potential utility of otolith shape analysis extends beyond that of stock identification. Catch misreporting by area/stock is common in some areas of the world; while otolith shape analysis would have insufficient discriminatory power to confirm the catch location of some stocks, only a handful of otoliths would have to be sampled from the catch to distinguish between, say, Gulf of Maine and Gulf of St. Lawrence cod. Studies of seal diet depend to a large extent on the species identification of otoliths collected from stomachs or scats (Murie and Lavigne 1985; Dellinger and Trillmich 1988). Otolith shape analysis could conceivably be used to help determine the area of feeding or test for size-selective prey mortality. Finally, archaeologists and paleontologists, who now use otolith shape to confirm species identity in fossil cores and archaeological digs, could extend their conclusions to include area of capture. In all of the above applications, the major advantage of otolith shape analysis is the requirement for only the otolith; neither the fish carcass nor body measurements are needed.

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Microhabitat Segregation of the Threatened Spotted Darter (*Etheostoma maculatum*) and Closely Related Orangefin Darter (*E. bellum*)

Richard K. Kessler and James H. Thorp

Water Resources Laboratory and Biology Department, University of Louisville, Louisville, KY 40292, USA

Kessler, R.K., and J.H. Thorp. 1993. Microhabitat segregation of the threatened spotted darter (*Etheostoma maculatum*) and closely related orangefin darter (*E. bellum*). Can. J. Fish. Aquat. Sci. 50: 1084–1091.

We examined differences in microhabitat use in sympatric populations of the closely related spotted darter (*Etheostoma maculatum*) and orangefin darter (*E. bellum*) (subgenus *Nothonotus*). Sample plots and underwater observations were employed to assess patterns of microhabitat use by both species. Quantitative sampling using a prepositioned electrofishing sampler demonstrated that species segregated by microhabitats. *Etheostoma maculatum* occupied areas of greater depth and larger, more diverse substrate sizes than *E. bellum* in July and October of 1991. Underwater observations also revealed species-specific differences in substrate use. *Etheostoma maculatum* was observed mostly under rocks whereas *E. bellum* was more general in substrate use. The narrow range of microhabitat use by *E. maculatum* may contribute to its status as a threatened species in Kentucky. During July 1991, an unusually high discharge month that year, males of both species occupied microhabitats of significantly higher velocities and larger substrate sizes than females. Our data suggest that opportunities for coexistence between these closely related darters are enhanced by microhabitat partitioning which is linked to behavioral and morphological differences.

Nous avons étudié les différences dans l'utilisation du microhabitat dans une population sympatrique de dards noirs (*Etheostoma maculatum*) et de dards «à nageoire orangée» (*E. bellum*) étroitement apparentés (sous-genre *Nothonotus*). Des parcelles d'échantillonnage et des observations sous l'eau ont été utilisées pour évaluer les modes d'utilisation du microhabitat par les deux espèces. L'échantillonnage quantitatif à l'aide d'une échantillonneur électrique préalablement mis en place a montré une ségrégation entre les deux espèces en fonction du microhabitat. *Etheostoma maculatum* occupait des zones plus profondes et des tailles de substrat plus grandes et plus diversifiées qu'*E. bellum* en juillet et octobre 1991. Des observations sous l'eau ont également révélé des différences spécifiques de l'espèce dans l'utilisation du substrat. *Etheostoma maculatum* était observé principalement sous les roches, tandis qu'*E. bellum* faisait une utilisation plus générale du substrat. L'utilisation étroite du microhabitat par *E. maculatum* pourrait avoir contribué à son état d'espèce menacée au Kentucky. En juillet 1991, qui s'est révélé un mois où le débit était inhabituellement fort cette année-là, les mâles des deux espèces ont occupé des microhabitats où la vitesse de l'eau et la taille du substrat étaient plus grandes que dans les microhabitats occupés par les femelles. Nous données indiquent que la possibilité de coexistence entre ces dards étroitement apparentés est augmentée par le fractionnement du microhabitat qui est lié à des différences comportementales et morphologiques.

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Resource partitioning is often cited as a mechanism that facilitates species coexistence in competitively driven systems (Schoener 1974, 1986; Bradshaw and Mortimer 1986), and therefore, analyses of such species interactions are crucial for comprehending community structure and functioning (Cody and Diamond 1975). Ross's (1986) review of niche partitioning in fishes indicated that most studies addressed species segregation along a food axis. Relatively few studies have focused on closely related, sympatric assemblages (Hlohowskyj and White 1983).

Habitat partitioning is one of the more important factors allowing coexistence of species (Rosenzwieg 1981). The way space is used is a crucial determinant of niche relations and population structure in many fishes (Gorman 1987). Benthic darters may partition either microhabitat, food, or both (Smart and Gee 1979; Paine et al. 1982; Englert and Seghers 1983; Hlohowskyj and Wissing 1986), but segregation in space is often especially important (Ross et al. 1987). Most darters of the genus *Etheostoma* (Percidae) have adapted to life in stream riffles and associated habitats where food availability is high and exposure to predators is low (Kuehne and Barbour 1983; Page and Swofford 1984). Substrate size and arrangement are among the more important microhabitat features for benthic organisms (Hynes 1970; Page 1983; Hlohowskyj and Wissing 1986; Thorp and Covich 1991). Riffles, which are characterized by high substrate heterogeneity (Hynes 1970), may allow coexistence of fish species through spatial partitioning (Page and Swofford 1984). Their small size may enable darters to partition space by differentially exploiting interstices in the substrate for food, shelter, and reproduction (Miller 1979).

We examined the use of microhabitat by two closely related, sympatric darters of the same subgenus, *Etheostoma* (*Nothonotus*), to determine whether spatial partitioning occurred. We define microhabitat as the suite of variables describing a site

occupied by an individual fish at any point in time (sensu Baltz 1990). A dual approach of macroscale (sample plots) and microscale (in situ behavioral observations) analyses was used to determine microhabitat use in both species. Our specific objectives were to (1) evaluate if use of space differed significantly within (sex specific) and between species, (2) identify factors contributing to microhabitat partitioning, and (3) ascertain whether patterns of microhabitat use by *Etheostoma maculatum*, the spotted darter, could contribute to its threatened status in Kentucky.

Materials and Methods

Species

Etheostoma bellum, the orangefin darter, is a deep-bodied, robust fish with a blunter snout than other species of its subgenus (Page 1983). It is endemic to the upper Green River drainage above the confluence of the Green and Barren rivers (Zorach 1968; Kuehne and Barbour 1983; Page 1983; Burr and Warren 1985; Fisher 1990) and may be locally abundant throughout its range. *Etheostoma bellum* typically inhabits shallow, clear riffles with gravel in smaller tributaries or riffle borders in the Green or Barren River proper (Zorach 1968; Kuehne and Barbour 1983; Page 1983; Burr and Warren 1986; Fisher 1990).

Etheostoma maculatum is a relatively deep-bodied fish with extreme lateral compression and a sharper snout than all but one species of its subgenus (Page and Burr 1991; Page 1983). The spotted darter inhabits swift, rubble riffles in medium to large streams (Zorach and Raney 1967; Stiles 1972; Kuehne and Barbour 1983; Page 1983; Burr and Warren 1986). Its distribution is sporadic, having disappeared from much of its former range (Page 1983), and it is considered a threatened species in Kentucky (Warren et al. 1986). Stiles (1972) compared the ecology of *E.* (*Nothonotus*) *maculatum vulneratum* with that of two other species (*Nothonotus*). Subsequently, *E. m. vulneratum* was elevated to *E. vulneratum*, the wounded darter (American Fisheries Society 1991).

Study Area

Russell Creek is a fifth-order tributary of the upper Green River, Green County, KY (85°29′48″W, 37°11′18″N) (average monthly discharge for the 1991 water year = 10.15 m^3/s; drainage area = 302.68 km^2; U.S. Geological Survey Preliminary Water Report 1992). Three sites on a lower reach of Russell Creek separated by approximately 500 m were sampled in July and October 1991. July was chosen to detect potential postspawning effects and October represented a nonreproductive period. We avoided sampling in the spawning period (late May to June) to avoid disturbing reproductive efforts of the threatened spotted darter. Data were collected in each site from a riffle and adjacent upstream and downstream runs or raceways (as defined by Winn 1958).

Sample Plot Characteristics

In all three sites, a 10-m-long section of each riffle and run was randomly chosen for sampling. Two transects were established across the width of the stream in each of these 10-m sections at 2.5 and 7.5 m from the upstream boundary. Three sample plots were established per transect for a total of 54 sample plots per month (3 sites × 3 sections × 2 transects × 3 plots).

Plot characteristics were measured in both seasons prior to quantifying fish density and distribution by using the following stratified transect sampling method. A 1-m^2 PVC-pipe frame delineated plots for estimation of microhabitat characteristics. Plots were established 1 m from either bank and at the middle of each transect to standardize sampling protocol between sites, to maximize collecting efforts (based on seining surveys), and to provide a comparison of left and right bank versus midstream effects (right and left banks were physically dissimilar within each site).

Physical characteristics of plots were based on measurements of both position within the stream and microhabitat variables within individual plots. Plot location from upstream to downstream was coded (1–6) based on the transect number. Current velocity near the bottom was measured using a Weather Measure model F581-B digital flow meter held approximately 5 cm off the bottom in the middle of each plot. Mean depth was estimated from the average of 50 measurements taken within a 1-m^2 grid (5 columns × 10 rows) placed on top of the sampling frame. Substrate roughness (or unevenness) was estimated from the mean standard deviation of the 50 depth measurements. This method is similar to Statzner et al. (1988), but standard deviations of depths for both rows and columns were computed by the technique used here. Substrate size was estimated visually based on a modified Wentworth scale (Cummins 1962). The following size ranges were observed: slate bedrock, 0 mm; silt, 0–0.05 mm; gravel, 0.05–2 mm; pebble, 2–16 mm; cobble, 64–250 mm; boulder, >250 mm. Substrate type was coded according to the two most prevalent size classes in decreasing order within a plot. The following substrate types (based on combined particle size of the two most prevalent size classes) and their rank were recorded from plots containing either study species: slate bedrock/gravel = 1; pebble/gravel = 2; pebble/cobble = 3; cobble/pebble = 4; boulder/pebble = 5; cobble/boulder = 6; boulder/cobble = 7. The presence or absence of vascular plants (mainly water willow, *Justicia sp.*), moss (*Fissidens sp.*), woody debris, and leaf packs was also recorded.

Fish Density and Distribution

Fish density and distribution were determined by collecting fish in all 54 plots on July 10–12 and October 18–20, 1991, with 18 prepositioned electrofishing samples (PES) (Weddle and Kessler 1993) (for similar methods, see Bain and Finn 1982, 1991; Bain et al. 1985; Peters et al. 1989) employed sequentially in the three sites. PES's were constructed of 2-cm (3/4 in.) PVC pipes and consisted of a 1-m^2 base and back-net (3-mm mesh); the frame was held in place with metal rods driven into the stream bottom (Weddle 1991). When current velocity was too great or debris too heavy, the back-nets were removed. When metal rods could not be driven, nylon ropes were first anchored on one bank. PES's were fastened onto these ropes and slid into position across the transect. The ropes were then weighted and anchored to the other bank. PES bases were electrified with alternating current from a generator (115 V, 600 W) on the stream bank. PES's were left undisturbed for ≥2 h before actual sampling began to allow fish more than adequate time to adjust to the PES's presence (Bain et al. 1985).

Downstream sites were sampled first, and plots were not approached until shocking began. Each plot was independently

shocked for about 15 s. Substrate was kicked during shocking to flush any benthic fish into the back-net. A 1.2-m, 3-mm-mesh seine was swept under the sampler after shocking to ensure collection of all fish. The two study species were immediately identified and placed in an aerated bucket. They were then sexed, and their standard lengths were measured to the nearest 0.1 mm. Both study species were placed in holding cages and allowed to recover before being released. Other species were either identified and released or preserved in 10% formalin and brought to the laboratory for future study.

Subsequent data have suggested that this fish sampling method is at least as effective as standard kick samples for collecting benthic fish (Weddle and Kessler 1993). Results of comparisons with adjacent kick samples suggest that PES's do not attract fish. The PES method may be preferred over kick samples because researchers can define microhabitats more precisely prior to sampling, and the likelihood of "fright-bias" (Bovee 1982) during the sampling process is reduced. Thirty-nine species representing nine families have been collected using the PES method in this and related studies (R.K. Kessler and G.K. Weddle, unpublished data).

Behavioral Observations

Underwater observations of individuals allowed us to determine more precisely if interspecific differences existed in the size of substrate used and the typical position of a fish (e.g., under or upon cobble). Although rarely used in the past, in situ behavioral observation of lotic fishes is gaining acceptance as an effective means for determining niche relations and population characteristics (Fausch and White 1981; Englert and Seghers 1983; Greenberg and Holtzman 1987; Greenberg 1988; Vogt and Coon 1990; Leidy 1992).

Using a snorkel and facemask, one of us (R.K.K.) observed individuals of both species on 12 separate days between July 18 and October 23, 1991, for a total of 25 h. All observations were made between 10:00 and 14:00 in areas (riffles) known previously to contain the highest densities of both species. A "focal animal observation method" (Altmann 1974) was employed in 1-m^2 plots examined consecutively across each of four new transects 2.5 m apart (i.e., they were not associated with the shocking transects described previously). Downstream transects were always observed first. Each plot was approached from downstream, and fish were observed individually for 1 min or until they swam away (whichever occurred first); this constituted one observation period. Individuals immediately reacting to the observer's presence were disregarded. Fish behavior was recorded for a total of 274 observation periods. The size of rocks used (modified Wentworth scale), arrangement of the rocks (i.e., stacked, overlapped, or flat), and the most typical orientation of individuals to the rocks (i.e., top, side, between, or under) were recorded for each observation period. Qualitative data on behaviors, such as intra- or interspecific interactions between the two study species, were also recorded.

Statistical Analyses

Both multi- and univariate statistical methods were used to determine intra- and interspecific patterns of resource use. One of the major problems in dealing with habitat relationships among stream organisms is that many habitat variables may vary concomitantly. To simplify this problem, principal components analysis (PCA) was used to construct a reduced coordinate system that better explained ecological relationships within the stream (Ludwig and Reynolds 1988). Factor analysis of all plots (n = 108) reduced variable redundancy (for protocol, see Pimental and Smith 1986). Data from plots (n = 54) were used to generate PCA components for each month. Only variables that loaded onto a component with an absolute value >0.400 were considered of significant importance to that component (Felley 1984; Matthews 1985). If a variable loaded onto more than one component, only the highest loading was applied to a component. The resultant display functioned as a two- or three-dimensional representation of multivariate environmental space. Mean factor scores were calculated for each species category (i.e., *E. bellum* or *E. bellum* males or females), and the centroids were plotted (sensu Matthews 1985b) to allow analyses of fish microdistributions within and between the two months.

Microhabitat similarity was derived using Schoener's (1968) index for degree of similarity. Intra- and interspecific similarity in microhabitat use was based on the percent total occurrence of a fish category within each habitat quadrant. Microhabitat similarity was considered high when the Schoener index was >0.600. Matthews (1985b) employed a similar method to determine percent habitat similarity of seven midwestern fish species.

Significance of ecological relationships established in PCA was tested using univariate methods. Systat software (Wilkinson 1989) was employed to run a series of one-way (species-specific) and two-way ANOVA's (species × sex) to test for significant separation of fish over single microhabitat variables. The nonparametric Kruskal–Wallis test was used to evaluate the influence of substrate type. Mean values for microhabitat variables were weighted according to the number of individuals of a species taken from a specific plot (i.e., measurements from within plots were assigned to each individual of a species occurring therein). Individual fish were considered independent data points (i.e., a plot with five spotted darters was treated as five observations) in multi- and univariate statistical analyses. This common approach is justified in part because the sample unit was sufficiently small so that within-plot physical factors (depth, velocity, and substrate type and roughness) were considerably more homogeneous than between-plot factors (especially bank versus channel or riffle versus run). Univariate methods were not used to test for between-month differences, as single variables (i.e., velocity and depth) related to varying discharges would have confounded results.

Behavioral observations were analyzed with an $R \times C$ contingency table and G-test (Sokal and Rohlf 1981) to determine if the two species used substrate in significantly different ways. The test was based on a combined analysis of species orientation and substrate size interaction (n = 10).

Results

Population Dynamics

We collected a total of 74 adult (≥1 yr, ≥30 mm SL; Raney and Lachner 1939) *E. bellum* (July = 47, October = 37) and 116 *E. maculatum* (July = 74, October = 42). The vast majority of both species were taken from riffle plots as opposed to upper and lower runs combined (*E. bellum* = 71 versus 19%, *E. maculatum* = 67 versus 33%). *Etheostoma bellum* was present in 27 of 108 plots (July = 16, October = 11) and *E. maculatum* was found in 49 plots (July = 29, October = 20). For both months combined, 51.5% of the total collection

TABLE 1. Factor loading of six original variables onto principal components for July and October 1991.

	PC I	PC II	PC III
	July		
Depth	−0.093	0.792	
Velocity	0.805	−0.243	
Substrate size	0.596	0.170	
Roughness	0.526	0.654	
Moss	0.651	−0.211	
Leafpack	−0.136	0.486	
% of variation	28.864	23.701	
	October		
Depth	−0.024	0.185	−0.866
Velocity	0.782	0.187	−0.110
Substrate size	−0.163	0.850	−0.092
Roughness	0.427	0.696	0.054
Moss	0.806	−0.122	−0.094
Leafpack	−0.242	0.132	0.829
% of variation	31.175	21.028	19.538

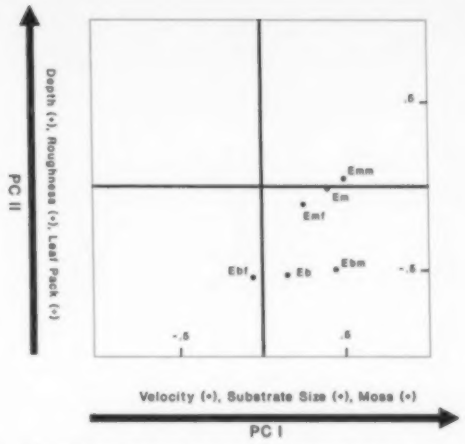

FIG. 1. Centroid ordinations for species categories based on mean factor scores in relation to two resource axes for July 1991. Em, *E. maculatum*; Emm, *E. maculatum* males; Emf, *E. maculatum* females; Eb, *E. bellum*; Ebm, *E. bellum* males; Ebf, *E. bellum* females. Parenthetic symbols denote (+) or (−) loadings.

TABLE 2. Mean factor scores of species categories used in July and October PCA (parenthetic numbers are standard deviations).

	PC I	PC II	PC III
	July		
E. bellum (f)	−0.052 (0.449)	−0.549 (0.449)	
E. bellum (m)	0.436 (0.594)	−0.508 (0.251)	
E. bellum	0.166 (0.519)	−0.531 (0.242)	
E. maculatum (f)	0.265 (0.726)	−0.117 (0.423)	
E. maculatum (m)	0.480 (0.635)	0.046 (0.516)	
E. maculatum	0.411 (0.664)	−0.007 (0.503)	
	October		
E. bellum (f)	0.193 (0.605)	0.021 (0.624)	0.100 (0.561)
E. bellum (m)	0.169 (0.433)	−0.099 (0.299)	0.309 (0.615)
E. bellum	0.179 (0.527)	−0.047 (0.472)	0.219 (0.602)
E. maculatum (f)	0.250 (0.446)	0.332 (0.486)	−0.036 (0.494)
E. maculatum (m)	0.337 (0.598)	0.254 (0.482)	−0.059 (0.595)
E. maculatum	0.304 (0.559)	0.284 (0.485)	−0.050 (0.540)

of *E. bellum* were taken from sample plots also containing *E. maculatum* (n = 18), while *E. maculatum* cooccurred with *E. bellum* 50% of the time. Fish densities tended to be higher for both species in July (mean ± SD: *E. bellum* = 0.85 ± 1.75 fish/plot, *E. maculatum* = 1.37 ± 2.08 (fish/plot) than October (*E. bellum* = 0.69 ± 1.85 fish/plot, *E. maculatum* = 0.78 ± 1.34 fish/plot).

Size varied between and within species. *Etheostoma bellum* was on average about 5% smaller than *E. maculatum* (mean SL's = 44.1 ± 6.49 and 46.4 ± 7.22 mm, respectively). The longest *E. bellum* was 59.5 mm SL whereas the largest *E. maculatum* was 71.6 mm SL; both of these males were smaller than the maximum length of 75 mm reported for both species (Page 1983). Male *E. bellum* (n = 42) and *E. maculatum* (n = 76) (months combined; mean = 46.2 ± 7.25 and 47.9 ± 7.99 mm, respectively) were larger than females (*E. bellum* (n = 42) and *E. maculatum* (n = 40) (mean = 42.0 ± 4.39 and 44.9 ± 6.34 mm, respectively) (ANOVA, F = 13.19, df = 199, $p < 0.001$).

Microhabitat Use

PCA reduced the number of original microhabitat variables from 12 to six and explained much of the variation within the data (Table 1). In July, two principal components (PC I and PC II) accounted for 52.6% of the total variance. In October, PC I, PC II, and PC III accounted for 71.7%.

Factor scores (Table 2) indicate that differences in microhabitat use existed both within (in July) and between species (July and October). Species centroids show that both species predominately inhabited shallow areas with fast current and relatively large, rough substrate in July (Fig. 1). *Etheostoma bellum* occurred in shallow water (mean depth ± SE = 12.55 ± 0.80 cm) over small (mean substrate size (coded) = 2.81 ±

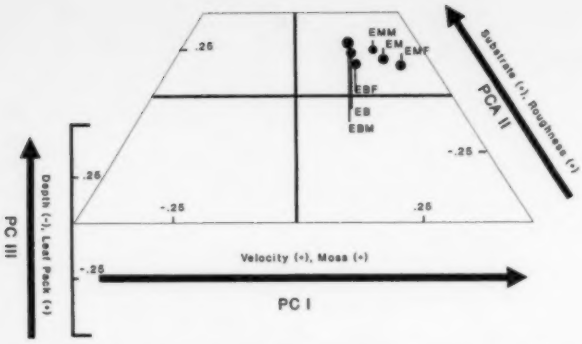

FIG. 2. Centroid ordination for species categories based on mean factor scores in relation to three resource axes for October 1991. Abbreviations and symbols as in Fig. 1.

0.16 cm), smooth substrates (mean roughness (coded) = 4.06 ± 0.16 cm). *Etheostoma maculatum* inhabited areas with deeper water (mean depth = 22.21 ± 1.15 cm) and larger (mean substrate size (coded) = 4.00 ± 0.21 cm), rougher substrates (mean roughness = 5.57 ± 0.24 cm). Intraspecific separations were also evident. Males of both species (n = 71) were found in areas with faster current (mean velocity = 0.75 ± 0.05 m/s) and larger (mean substrate size (coded) = 3.79 ± 0.28 cm), rougher substrate (mean roughness = 5.13 ± 0.30 cm). Females (n = 50) occupied areas with slower current (mean velocity = 0.56 ± 0.06 m/s) and smaller (mean substrate size (coded) = 2.95 ± 0.24 cm), smoother substrate (mean roughness = 4.415 ± 0.22 cm).

In October (Fig. 2), both species continued to use microhabitats with fast current, and *E. bellum* remained in shallow areas. *Etheostoma bellum* was found in shallower water (mean depth ± SE = 13.90 ± 0.87 cm) and smaller (mean substrate size (coded) = 2.89 ± 0.12 cm), smoother substrate (mean roughness = 4.15 ± 0.23 cm) than was *E. maculatum*. *Etheostoma maculatum* continued to occupy sites with deeper water (mean depth = 17.64 ± 0.92 cm) and larger (mean substrate size (coded) = 3.41 ± 0.15 cm) rougher substrate (mean roughness = 5.08 ± 0.18 cm). The distribution of *E. maculatum* was not strongly affected by depth in either season. Within-species separations were not apparent in October.

The inter- and intraspecific differences of *E. bellum* and *E. maculatum* shown by PCA are supported by univariate one-way (species-specific differences) and two-way ANOVA's (species × sex). In July (Fig. 3), interspecific differences existed for depth (ANOVA, $F = 31.86$, df = 120, $p < 0.001$), substrate size (based on substrate code; Kruskal-Wallis, $\chi^2 = 16.30$, df = 120, $p < 0.001$), and substrate roughness (ANOVA, $F = 49.18$, df = 120, $p < 0.001$). In both months, substrate size and depth were significantly correlated with roughness (Pearson correlation coefficient = 0.455 and 0.256, respectively, df = 107, $p < 0.05$). Sex-specific differences in velocity and substrate size used (Fig. 4) also occurred (ANOVA, $F = 11.04$, df = 120, $p < 0.005$ and Kruskal–Wallis, $\chi^2 = 16.48$, df = 120, $p < 0.001$, respectively).

In October, *E. bellum* occupied areas with shallower water (ANOVA, $F = 7.35$, df = 78, $p < 0.01$) and smaller (ANOVA, $F = 6.68$, df = 78, $p < 0.05$), smoother substrate (ANOVA, $F = 16.05$, df = 78, $p < 0.005$) than did *E. maculatum*. Significant intraspecific differences in October were absent.

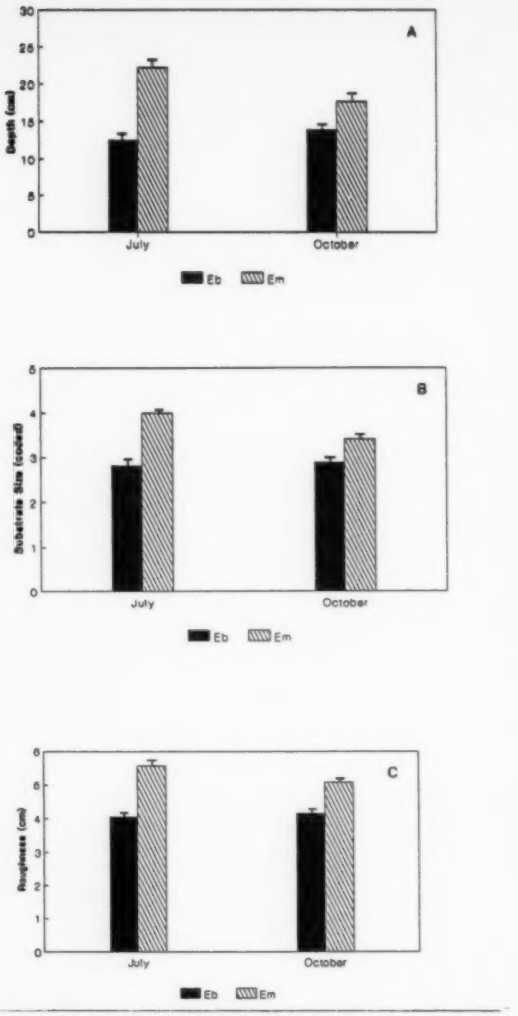

FIG. 3. Significant species-specific mean differences (+1 SE) in (A) depth, (B) substrate size, and (C) substrate roughness for July and October 1991. Eb, *E. bellum*; Em, *E. maculatum*.

TABLE 3. Degree of habitat similarity (D) of species and gender categories based on percent total occurrences in each PCA habitat quadrant for July and October 1991. Minimum = 0; maximum = 1.00.

	E. bellum (m)	*E. bellum* (f)	*E. maculatum* (m)
	July		
E. bellum (f)	0.729	—	
E. maculatum (m)	0.535	0.440	—
E. maculatum (f)	0.577	0.625	0.787
	October		
E. bellum (f)	0.801	—	
E. maculatum (m)	0.665	0.608	—
E. maculatum (f)	0.551	0.563	0.861

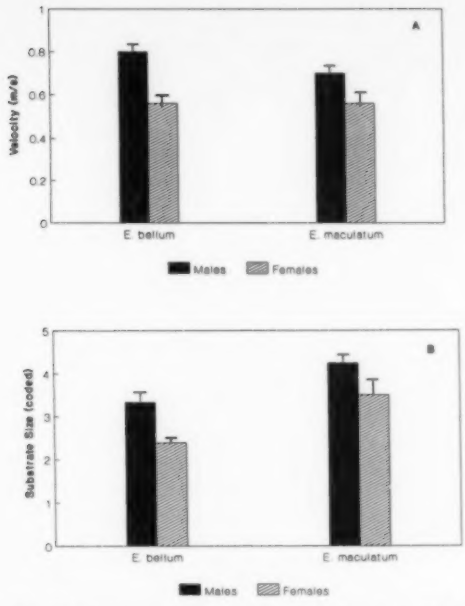

FIG. 4. Significant sex-specific mean differences (+1 SE) in (A) velocity and (B) substrate size for July 1991.

Microhabitat similarity within species was high (>0.600) for both months, and overlap within species was correspondingly greater than that found between species (Table 3). Microhabitat use between sexes of opposite species was similar for both seasons (e.g., spatial positioning of males of *E. bellum* were comparable with males of *E. maculatum* for both months), but the concordance was always less than between sexes of the same species.

Behavioral Observations

Etheostoma bellum and *E. maculatum* used substrate in significantly different ways (*G*-test, $\chi^2 = 31.26$, df = 9, $p < 0.001$). *Etheostoma bellum* ($n = 105$) exhibited a wide range of orientations to a variety of substrate sizes, while *E. maculatum* ($n = 169$) was almost exclusively (86.4%) observed under rocks. More specifically, *E. bellum* was most frequently observed nestled between pebbles or holding to the side of a large pebble or cobble.

The arrangement of substrate may be particularly important to *E. maculatum*. In about half the 86 occurrences of *E. maculatum* under cobble, individual rocks were stacked or overlapping in a way that created sufficient refugia for darters. At other times, *E. maculatum* used open areas under single, large cobbles or boulders. Such open areas can be created by an irregular bottom or when loose gravel is swept out from under a large rock. Arrangement appears to be even more important to *E. maculatum* when using smaller substrates. Of the 35 times *E. maculatum* was observed under pebbles, the rocks were arranged in a stacked or overlapping fashion 83% of the time.

Discussion

Data suggest that interspecific microhabitat partitioning by *E. bellum* and *E. maculatum* occurred along gradients of substrate roughness, substrate size, and depth in two months and that intraspecific separation along gradients of velocity and substrate size occurred in July. Of the significant differences in depth, substrate size, and roughness that existed between species in one or both seasons, it appears that the latter two factors were the most important. Substrate roughness is a measure equivalent to the landscape ecology term "contrast" (Forman and Godron 1986). High measures of roughness correspond to highly irregular bottoms with complex substrates having abundant interstitial space. Our results suggest that substrate diversity may be more important than substrate size in determining the distributions of these two darters. Weddle (1991) expressed a similar conclusion for *E. rafinesquei*, the Kentucky darter.

Both study species use substrate to maintain position, to rest, for refugia, and for reproduction; however, *E. bellum*'s more robust, streamlined body and large pectoral fins appear better suited for exposed benthic microhabitats. Although the orangefin darter is reported to occur primarily over gravel (Kuehne and Barbour 1983), we observed it to be highly diverse in substrate use. Such flexibility should encourage coexistence of species (Chesson and Rosenzwieg 1991). While *E. bellum* may exploit space under rocks, it certainly relies on substrate unevenness to maintain its position in fast-flowing streams. *Etheostoma bellum* usually wedged itself between rocks, holding station with its pectoral fins, and often arched its body (perhaps allowing the fish to maintain closer contact with the rock). Similar behaviors have been observed in other darters (Matthews 1985a) and crayfish (Maude and Williams 1983) in response to rising water velocity. Matthews (1985a) concluded that microhabitat partitioning in *Percina roanoka* and *E. flabellare* reflected their disparate abilities to hold station in current. He found that morphological and behavioral differences enhanced these abilities but that pectoral fins were unimportant for holding position on smooth substrate.

Etheostoma maculatum, although closely related to *E. bellum*, has dramatically different space requirements, as it uses areas under rocks for both reproduction and refugia (Winn 1958; present study). The spotted darter is a more laterally compressed species, which allows it to fit within crevices and under rocks. *Etheostoma maculatum* typically favors substrates with high degrees of roughness, such as cobble and boulders (Kuehne and Barbour 1983; Page 1983). Substrate size is apparently unimportant when partial burial eliminates space under rocks (Stiles 1972). Our research indicates that arrangement and diversity of substrate are important to *E. maculatum* and may permit use of pebbles and other small rocks.

Our observations that these two species occupied areas of different average depths are supported by the results of previous studies. *Etheostoma bellum* has been reported from shallow riffle margins near the banks (Kuehne and Barbour 1983; Fisher 1990). Furthermore, a greater percentage of *E. maculatum* than *E. bellum* were found in deeper upstream and downstream runs (present study). The distribution of *E. maculatum* was not strongly related to depth in either July or October according to PCA.

July distributions of both species could have reflected post-spawning, interhabitat movements, or the latent effects of spawning (see Page 1983) because both darters are known to spawn until late June (Raney and Lachner 1939; Zorach 1968; Floyd et al. 1984; Fisher 1990), and we found no gravid females of either species.

Previous studies of the subgenus *Nothonotus* have recognized the importance of both size- (Greenberg 1988) and age-

specific (Fisher 1990) microhabitat segregation. The intraspecific segregation of substrate size and velocity in both species may have resulted from sex-specific preferences, but it is probably related more to sexually dimorphic sizes. Sexual dimorphism in body size is common for darter species with territorial males (Page 1983). Both *E. maculatum* (Raney and Lachner 1939) and *E. bellum* (Fisher 1990) are considered territorial, and we found significant sex-specific size differences within each species. Segregation of habitat by gender may reflect the ability of larger males to withstand stronger currents (Page and Swofford 1984). This hypothesis is supported by our data showing that the greatest segregation by gender was in July, which was characterized by unusually high discharges.

Male and female spotted darters (Raney and Lachner 1939) and at least male orangefin darters (Fisher 1990) are territorial, a factor that can sometimes contribute to coexistence of two species having strong dietary overlap (Thorp 1976), such as *E. bellum* and *E. maculatum* (R.K. Kessler, unpublished data). The closely related *E. vulneratum* is also quite aggressive in defending sites against both congeneric and conspecific individuals (Stiles 1972). Agonistic interactions, even among territorial species, may typically be rare and aperiodic events in some aquatic taxa. We were fortunate, therefore, to observe an agonistic encounter in late August when a male *E. bellum*, reacting to the observer's presence, swam under a rock occupied by a large *E. maculatum* male; the intruder was immediately chased away by the resident spotted darter. Territoriality, as a form of interference competition, does not necessarily involve exploitation competition for limiting resources (Schoener 1986); and in the case of orangefin and spotted darters, it may simply reinforce microhabitat segregation.

Behavioral segregation of two or more species may occur when physical separation increases fitness by enhancing resource availability, reducing interference competition, or lowering noncompetitive mortality from biotic or abiotic factors (Brown and Rosenzwieg 1986). Spotted darters clearly favor sites with complex substrate arrangement. In contrast, orangefin darters, while perhaps preferring some of the same sites, exhibit a flexible behavior that allows them to exploit habitats of marginal value for spotted darters. This behavioral plasticity by orangefin darters could contribute to coexistence of these ecologically similar fishes (cf. Chesson and Rosenzwieg 1991).

The microhabitat limitations of *E. maculatum* shown here may help explain its threatened status in Kentucky. Kuehne and Barbour (1983) and Page (1983) noted the disappearance of the spotted darter from much of its range and reductions in densities at most other sites. The spotted darter's downfall may be its preference for areas with large, loose, and rough substrates which provide ample interstitial space for refugia and reproduction. Excessive siltation or sedimentation resulting from careless land-use practices (e.g., some associated with agriculture, logging, and mining) may significantly reduce availability of these sites rendering them "useless" (Danielson 1991) to spotted darters. This conclusion is borne out by our sampling efforts throughout the upper Green River drainage of south-central Kentucky. While abundant at a few sites, *E. maculatum* was never found in areas with silt-covered rocks and only rarely occurred where substrates were packed (R.K. Kessler, personal observation). The former are unsuitable for its egg-clumping, reproductive behavior, and a reduction in such sites would increase the spotted darter's susceptibility to current velocity. Ross et al. (1992) demonstrated the importance of refugia from fast currents for winter survival of *E. rubrum* (*Nothonotus*).

In summary, it appears that spatial segregation is a key contributor to coexistence of these two closely related darters in Russell Creek. Interspecific microhabitat partitioning, which was enhanced by behavioral and morphological differences, occurred along gradients of depth, substrate size, and substrate roughness in both months. Intraspecific differences in microhabitat use (velocity and substrate size) were evident in July. Future research, especially thorough diet anlysis and analyses of habitat preferences based on habitat availability, would improve our understanding of relationships among darter assemblages and their environment. Finally, it is imperative that drainage areas supporting the remaining populations of *E. maculatum* be protected from ongoing processes leading to excessive siltation and sedimentation if the spotted darter is to survive.

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Chehalis River Disease: A Unique Gill Disease of Salmonids

G.C. Newbound,[1] D.J. Speare, and K.L. Hammell

Department of Pathology and Microbiology, Atlantic Veterinary College, University of Prince Edward Island, 550 University Ave., Charlottetown, P.E.I., Canada C1A 4P3

M.L. Kent

Department of Fisheries and Oceans, Biological Sciences Branch, Pacific Biological Station, Nanaimo, B.C., Canada V9R 5K6

V.E. Ostland

Department of Pathology, Ontario Veterinary College, University of Guelph, Guelph, Ont., Canada N1G 2W1

and G.S. Traxler

Department of Fisheries and Oceans, Biological Sciences Branch, Pacific Biological Station, Nanaimo, B.C., Canada V9R 5K6

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Epidemic mortalities of chinook salmon alevins (*Oncorhynchus tshawytscha*) have occurred annually at a freshwater hatchery at the Chehalis River, British Columbia, Canada, since 1982. Previous work has identified branchial epithelial hyperplasia as the predominant pathological change. Our initial work lead us to test the hypothesis that Chehalis River disease (CRD) was actually a novel form of bacterial gill disease (BGD). Histological and ultrastructural observations indicated that CRD-affected gills harboured a bacterial biofilm apparently complexed with particulate iron prior to and during clinical CRD. In one instance, extracellular viruslike particles were observed. Large numbers of a mixed bacterial population were recovered from diseased gills. *Flavobacterium* sp. and *Pseudomonas fluorescens* were considered the predominant isolates from the 1990–91 outbreak. However, indirect immunofluorescence of gills with anti-*Flavobacterium branchophila* serum failed to detect antigenically similar bacteria on the gills of CRD-affected fish. Chemotherapeutic treatment of both naturally and experimentally infected fish was successful, thereby confirming the infectious nature of the disease. We conclude that CRD is a form of BGD, but the significance of the branchial viruslike particles and the presence of particulate iron has not yet been established.

Depuis 1982, des épidémies mortelles frappent annuellement les alevins de saumon chinook (*Oncorhynchus tshawytscha*) dans une écloserie d'eau douce de la rivière Chehalis, en Colombie-Britannique, Canada. Des travaux antérieurs ont révélé que la principale manifestation pathologique était une hyperplasie épithéliale branchiale. Nos travaux initiaux nous ont amenés à vérifier l'hypothèse que la maladie de la rivière Chehalis (MRC) soit en réalité une forme de bactériose des branchies (BB). Des observations histologiques et ultrastructurales ont indiqué que les branchies affectées par la MRC étaient enduites d'une biofilm bactérien apparemment complexé avec du fer particulaire avant et pendant le stade clinique de la MRC. Dans un cas, on a observé des particules extracellulaires d'apparence virale. On a isolé des branchies affectées une abondante population bactérienne mixte. *Flavobacterium* sp. et *Pseudomonas fluorescens* ont été considérés comme les isolats prédominant au cours de la poussée de 1990–1991. Toutefois, une étude par immunofluorescence indirecte des branchies avec un sérum anti-*Flavobacterium branchophila* n'a pas permis de déceler des bactéries antigéniquement semblables à cette espèce sur les branchies touchées par la MRC. La chimiothérapie appliquée à des poissons affectés naturellement et expérimentalement s'est révélée un succès, confirmant ainsi la nature infectieuse de la maladie. Nous avons conclu que la MRC est une forme de BB, mais la signification des particules d'apparence virale dans les branchies et de la présence du fer particulaire n'a pas été établie.

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Epidemic mortalities of chinook salmon (*Oncorhynchus tshawytscha*) alevins have occurred annually since 1982 at a government hatchery located on the Chehalis River, Upper Fraser Valley, British Columbia, Canada. Traxler and Rankin (1987) showed that the disease, referred to as Chehalis River disease (CRD), could be horizontally transmitted to several strains of chinook salmon and to at least one other species of salmonid, the chum salmon (*Oncorhynchus keta*). Branchial epithelial hyperplasia has been the only change described in alevins with CRD (Traxler and Rankin 1987). These workers also noted that other organs and structures appeared normal. It was proposed that CRD was caused by an unidentified virus because neither parasitic nor bacterial agents were identified (Traxler and Rankin 1987).

Several factors may contribute to outbreaks of CRD at the Chehalis River Hatchery. Withler (1987) demonstrated that the

[1]Author to whom correspondence should be addressed.

Harrison River chinook salmon strain is the strain most susceptible to CRD. Chinook production is largely devoted to this strain because it contributes heavily to the Canadian commerical and sport fisheries (Harding 1987). There are differences in water chemistry between the Chehalis River Hatchery and other hatcheries in British Columbia unaffected by CRD. However, modifications of these indices did not significantly alter mortalities attributed to CRD (Alderdice and Rombough 1987).

Based on a review of histological specimens of CRD-affected fish (collected from 1982 to 1988), the appearance of bacterial microcolonies on the surface of morphologically altered gill tissue empirically suggested that CRD is a gill disease associated with bacteria. The present investigation tests this hypothesis by the following methods: (1) a histopathological retrospective study of CRD-affected fish, (2) a histopathological study of sequentially sampled fish during the 1989–90 CRD outbreak to determine temporal relationships between presence of bacteria, morphologic branchial change, and clinical expression of CRD, (3) ultrastructural studies of affected branchial tissue, (4) bacteriological culture of affected gill tissue during the 1990–91 outbreak, and (5) testing the efficacy of chemotherapeutic treatments known to be effective against other forms of BGD on natural and experimentally induced infections.

Materials and Methods

Sample Collection

A retrospective study of the range of gill changes in alevins dying from CRD, from 1982 to 1988, was undertaken. Sagittal sections, stained with hematoxylin and eosin, from 100 clinically affected alevins were compared with sections from 40 clinically normal alevins collected from the same hatchery during the same period.

Sequential pathology of alevins from the Chehalis River Hatchery that developed CRD during the period from December 1989 to February 1990 was investigated using the following sample protocol. Thirteen Heath trays each containing approximately 5000 chinook alevins were arbitrarily selected from a total of 581 trays. From each tray, six alevins were arbitrarily sampled weekly for 10 wk and processed for light and/or electron microscopy. At the time of onset of clinical CRD, arbitrarily selected fish displaying typical clinical signs were sampled from an additional five trays.

Preparation of Samples

For light microscopy, entire fish were fixed in Davidson's solution (Humason 1979). Samples were then routinely prepared for histology, and 6 μm-thick sagittal sections of whole fish were stained with hematoxylin and eosin and examined. Appropriate sections were also stained with Gram, Perl's, or periodic acid – Schiff stains (Humason 1979).

For transmission and scanning electron microscopy (TEM and SEM), gill arches were dissected from alevins killed with an overdose of MS-222 (tricaine methanesulfonate) and immediately placed in cold 2.5% gluteraldehyde in 0.1 M phosphate buffer. Tissues were postfixed in 2% osmium tetroxide in phosphate buffer and dehydrated in a graded series of ethanol. For TEM, samples were cleared in propylene oxide, embedded in Epon 812, and thin sections (60–90 nm) cut and double stained with uranyl acetate and lead citrate. Sections were examined using a JEOL 100S transmission electron microscope. For SEM, dehydrated samples were critical point dried with CO_2 using a Tousomis Samodri PVT-3, mounted on aluminum stubs, and sputter coated with gold/palladium using a Technics Hummer 6 coater. Specimens were examined using a JEOL JSM-35 scanning electron microscope.

Bacteriology

During the 1990–91 outbreak, five fish showing clinical signs of CRD were arbitrarily selected from each of 10 Heath trays and killed with an overdose of MS-222. The first gill arch from the left side of each fish was used to confirm CRD by examination of a wet mount preparation. If a positive diagnosis was obtained, all of the gill arches from the right side of the fish were removed and placed in 3 mL of sterile 0.1% peptone in distilled water. The gill tissue was manually crushed, 10-fold dilutions were prepared to 10^{-5}, and 0.1 mL aliquots were spread onto individual plates of cytophaga agar containing 1.5% agar (1.5% CA; Anacker and Ordal 1959). For primary isolation, all plates were incubated aerobically at 15°C for 7 d. Cultures were observed daily for growth.

The most predominant bacterial isolates, based on colony morphology, were subcultured and maintained on 1.5% CA for further characterization based on the criteria of Holmes et al. (1984) and Palleroni (1984) using previously described methods (Ostland et al. 1989). Unless otherwise indicated, we used commercially available media in all tests. Cultures were incubated aerobically at 18°C for 7 d or longer, depending on the temperature.

Further identification was performed with the API 20E system as outlined by the manufacturer (API Produits de Laboratoire Ltee., St. Laurent, Que). Duplicate strips were used for each isolate; one strip was developed at 48 h postinoculation and the other at 14 d.

Indirect Immunofluorescence of CRD-Affected Gills

An indirect fluorescent antibody test (IFAT) was used to determine whether antigenic similarity existed between the bacteria present on the surface of the CRD-affected gills and the filamentous bacteria recovered from naturally occurring outbreaks of bacterial gill disease (BGD) in Ontario. Briefly, sagittal sections of CRD-affected whole chinook alevins were mounted on slides previously coated with Aptex (3-aminopropyltriethoxysilane, Sigma Chemicals, St. Louis, MO). The sections were deparaffinized in xylene and rehydrated through a series of graded alcohol baths and held in distilled water prior to immunohistochemical staining. Rabbit antiserum was previously raised against *Flavobacterium branchiophila*, a causative agent of BGD in both Ontario and Japan (anti-LAB4a serum (Ferguson et al. 1991) anti-ATCC 35036 serum (Wakabayashi et al. 1989), respectively). Sections were incubated in the primary antisera (1/1000 dilution) for 1 h at room temperature in a humid chamber, washed three times (5 min each) in phosphate-buffered saline (PBS), and then counterstained with a 1.6% eriochrome black (Sigma Chemicals) solution (in PBS) for 30 s. After a quick rinse in PBS, all sections were labelled with a commercially available FITC conjugated goat anti-rabbit H + L IgG serum (Kirkgard Perry Laboratories) for 30 min, followed by three PBS washes as described above. Coverslips were mounted using a sodium carbonate/bicarbonate-buffered glycerol prepared in distilled water (pH 9.1). Paraffin-embedded healthy gills and diseased gills from a naturally occurring outbreak of salmonid BGD in Ontario served as controls.

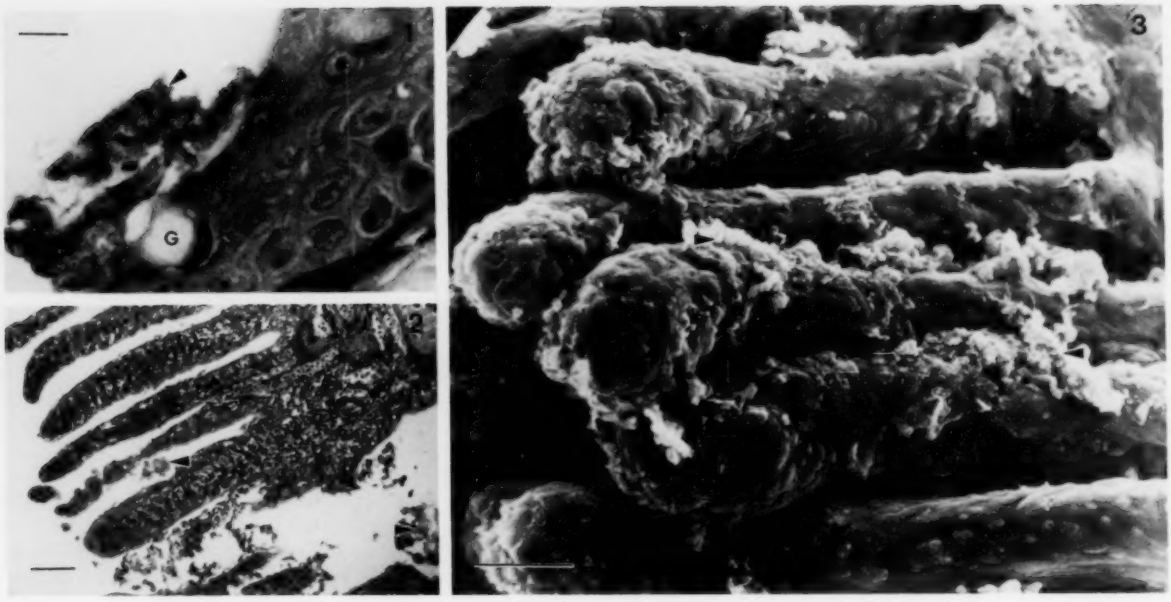


FIG. 1–3. *Oncorhynchus tshawytscha*. Fig. 1. Subacute erosive dermatitis. Arrowhead = bacterial biofilm; G = goblet cell. Bar = 4 μm. Fig. 2. Severe branchial lamellar and filamental fusion. Arrowheads = bacterial microcolonies. Bar = 375 μm. Fig. 3. SEM of fused branchial lamellae with associated bacterial microcolonies and environmental debris (arrowheads). Bar = 60 μm.

Treatment of Naturally Infected Alevins

Three Heath trays containing approximately 4000 chinook alevins each were diagnosed as having CRD by clinical signs and by examination of wet mount preparations of gills. Alevins from each of the three trays of moribund fish were pooled and then volumetrically divided equally into nine clean trays. The nine trays were placed, three trays per stack, in a series of three treatment stacks separated from the rest of the hatchery stacks.

The treatment stacks consisted of three adjacent racks that could each contain up to eight Heath trays. Each rack had a separate water supply originating from the same header tank. The water flow to each of these stacks ws 11 L/min, and the inflow was gavity-fed well water. The water flow through the racks was similar to most Heath tray systems, in that the entire inflow of water went into the top tray, while effluent from this tray became the inflow for the tray below.

Three identical plastic totes (101 × 68 × 56.5 cm, volume 388 L) were used as treatment vessels. These plastic totes were filled with well water, aerated with large air stones, and the appropriate amount of chemotherapeutant was added. Chloramine T (sodium para-toluenesulphonchloramide, Syndel Laboratories Ltd., Vancouver, B.C.) was added to the first tote to give a final concentration of 8.5 ppm (3.3 g), and Hyamine 1622 (benzalkonium chloride, Argent Chemical Laboratories, Richmond, B.C., 50% solution) was added to the second tote to give a final concentration of 2 ppm (1.5 mL). No chemotherapeutants were added to the third (control) tote. Treatment consisted of placing entire Heath trays into one of the three appropriate totes for two, 1-h baths 48 h apart. Subsequent to each treatment, the trays were replaced in the treatment stacks in the same order as pretreatment.

Six alevins were arbitrarily selected from each tray for histopatholological examination prior to the first treatment, after both treatments, and 1 wk after the second treatment. The selected alevins were preserved in Davidson's solution and prepared for histological examination as previously described.

Two to four fish were arbitrarily sampled weekly from the surviving population, and gills were examined by histological and wet mount preparations.

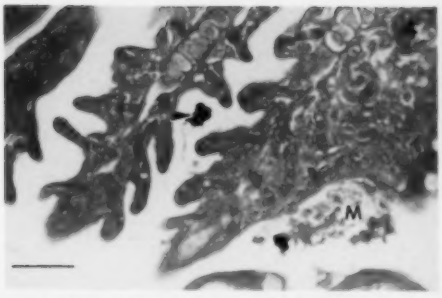
Treatment of Experimentally Induced Infections

Experimental transmission of CRD was carried out in a manner as described by Traxler and Rankin (1987). Briefly, at three different times during the alevin-rearing period, the apparently healthy populations in three or four trays, each tray containing approximately 6000 fish, were exposed to the effluent from CRD-positive trays for a period of 24–48 h. Following exposure, fish from these trays were pooled and volumetrically divided into nine or 12 equal groups and placed in separate Heath trays. These trays were randomly placed in the top three or four slots of the three treatment stacks and assigned to receive control, Chloramine T, or Hyamine baths. The trays were treated with the appropriate chemotherapeutant as described for the naturally infected fish.

Histopathological samples were taken from trays of healthy alevins subsequent to both treatments, and 1 wk subsequent to the final treatment. Surviving fish were also monitored weekly using wet mount and histological preparations.

Statistical Analysis

The experimental unit for the experimentally infected fish, on which statistical analyses were based, was the tray within the treatment stacks. The tray was the smallest aggregate of individuals that was randomized to treatment groups and on which the outcome of mortality was measured.



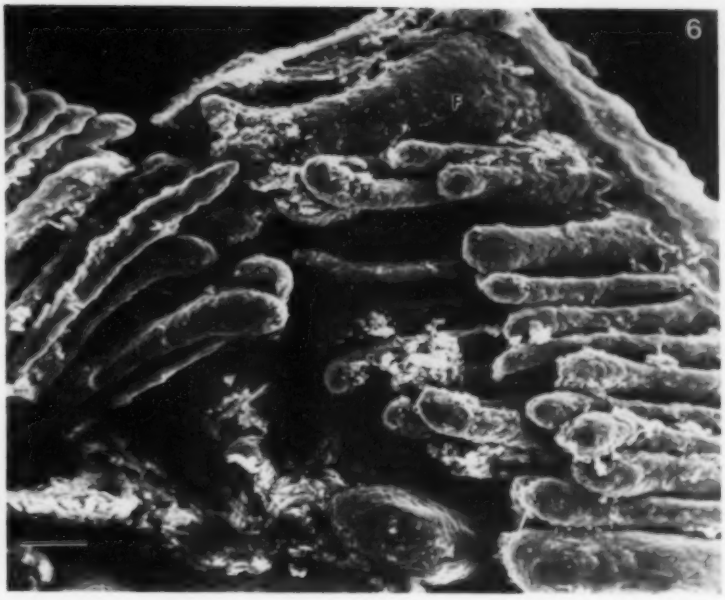


FIG. 4–6. *Oncorhynchus tshawytscha*. Fig. 4. Branchial bacterial microcolony with associated iron particles. Arrowhead = particulate iron; M = microcolony; Perl's iron stain. Bar = 200 μm. Fig. 5. Bacterial microcolony associated with normal branchial tissue. Arrowhead = bacterial microcolony. Bar = 650 μm. Fig. 6. SEM of CRD-affected hemibranch with extensive, severe lamellar and filamental fusion (F). Bar = 100 μm.

Treatment trials were run on four occasions throughout the 1990–91 outbreak with one tray in each of the three treatment groups (Chloramine, Hyamine, and control) during the first trial, three trays in each treatment group during the second trial, and four trays in each treatment group in both the third and fourth trials. Tray position within the treatment stacks and time of treatment during the outbreak (i.e., first versus sixth week) were potential confounders of the treatment effect. It was possible that lower trays in the treatment stacks would receive a higher pathogen load from the higher trays, and unobserved changes in the pathogen virulence or environmental conditions during the course of the outbreak could have affected tray mortality rates.

Due to the uneven numbers of trays in the trials, the fourth trays in the third and fourth trials were not included in the initial statistical analyses. The lone treatment tray in the first trial was not used in any statistical analyses because the confounding variables could not be evaluated.

Five null hypotheses were tested:

(1) There was no difference in mortality rates among the top three tray positions (controlling for treatment). This was tested using a two-way analysis of variance (ANOVA).

(2) There was no difference in mortality rates among different trials (controlling for treatment). This was tested using a two-way ANOVA.

(3) There was no interaction between treatment and tray position on mortality rates. This was tested within the ANOVA from 1.

(4) There was no interaction between treatment and trial on mortality rates. This was tested within the ANOVA from 2.

(5) There was no difference in mortality rates among different treatments. This was tested by comparing the mortality means of the 11 trays per treatment group using a one-way ANOVA.

The Minitab statistical package was used for analysis of the data (Ryan 1985). The Student–Newman–Keuls test was used to compare treatment means once a difference had been detected (Glantz 1987).

Results

Retrospective Study

Consistent changes were found in clinically affected fish from all outbreaks of CRD investigated. Changes were limited to the skin, gills, and the epithelium lining the branchial cavity. A range of mild to severe skin and dermal lesions were observed. Pattern analysis of the skin changes fulfilled the criteria for subacute erosive superficial bacterial dermatitis (Fig. 1). A biofilm composed of short- to medium-length Gram-negative rods and long filamentous Gram-negative bacterial rods occurred together at the surface of the lesions.

Gill lesions included chronic hyperplastic bacterial branchitis with mucous cell metaplasia. The degree of lamellar and filamental fusion of the gill was often severe (Fig. 2 and 3). The presence of individual necrotic lamellar epithelial cells was characteristic of the changes. Microcolonies of mixed Gram-negative rods were spatially associated with sites of gill damage. Bacterial microcolonies were often present in direct association with epithelial surfaces; otherwise, they were viewed within accumulations of mucus (Fig. 3). Large amounts of Perl's positive refractile granules, likely representing particulate iron, were present within this mucous film (Fig. 4).

Sequential Study

Clinical signs similar to previous outbreaks of CRD were reported by hatchery workers on the sixth week posthatch in two of the 13 sequentially sampled trays.

TABLE 1. Sequential prevalence (number positive/number sampled) of iron, bacteria and lesions (CRD) in gills from two trays of chinook salmon alevins that developed CRD.

	Week of sample posthatch						
	1	2	3	4	5	6	7
Iron	2/9	4/12	6/11	3/12	3/12	8/11	8/9
Bacteria	1/9	3/12	4/11	5/12	9/12	10/11	9/9
CRD	0/9	0/12	0/11	0/12	2/12	8/11	9/9

The sequential prevalence of bacteria, iron particles, and lesions from the two affected, sequentially sampled trays is summarized in Table 1. Briefly, bacteria and iron particles were present on the gill tissues of alevins as early as 1 wk posthatch. Although the quantity of entrapped iron did not appear to change before CRD outbreaks occurred, the prevalence of branchial bacterial colonization increased. On the gills of fish in both trays, coalescing microcolonies of bacteria formed a biofilm that closely preceded the development of CRD (Fig. 5). In comparison, only small numbers of nonaggregated bacteria could be found on the gills of alevins sampled from trays that did not develop CRD. Particles of iron were found on approximately 50% of the alevins from affected trays, and an erosive bacterial dermatitis was present on approximately 15% of all affected alevins.

The morphologic changes that developed in the CRD-positive sequentially sampled fish were the same as those noted in the retrospective study. Additionally, the range of lesions affecting the gills of the alevins from the five trays sampled as they exhibited clinical signs of CRD were also the same and will not be described separately. In comparison, no gill changes were noted in the alevins from the 11 sequentially sampled trays that did not develop CRD.

The sequential pathological changes in gill tissue had the following general features. Bacterial colonization, with the development of microcolonies, preceded tissue changes by approximately 1 wk. Gill tissue changes preceded the onset of clinical disease during a similar period. Structural alterations, including lamellar fusion and hyperplasia of filament epithelium, first developed along the distal aspects of filaments. Mucous cell metaplasia and migration of mucous cells out onto lamellae, accompanied by mucus hypersecretion, developed along with hyperplasia. Lamellar edema was frequently noted. Chronic changes, noted in alevins sampled 1 wk after the onset of disease, were characterized by extensive filament fusion and filament epithelial hyperplasia. In some dramatic cases, fusion occurred along the entire length of the filaments (Fig. 6). This was accompanied by large numbers of bacteria and exfoliated epithelial cells. Multifocal areas of erosive superficial bacterial dermatitis had sporadically developed on CRD-positive alevins throughout the course of the outbreaks.

Ultrastructural Pathology

Epithelial and chloride cells in the regions of the gill colonized by bacteria exhibited characteristic degenerative changes. Plasma membrane changes included microridge degeneration and exfoliation of membrane-bound vesicles (Fig. 7). Organelle changes included variable to pronounced dilation of the cytocavitory network and mitochondria. Similar but more pronounced changes were noted in necrotic cells that also exhibited changes affecting the nucleus and intercellular junctional complexes.

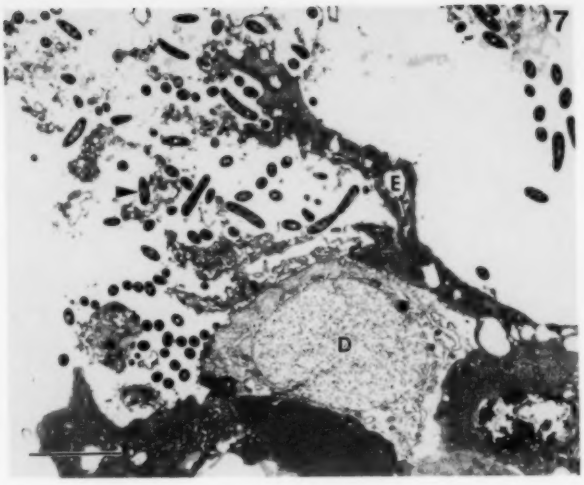


FIG. 7. *Oncorhynchus tshawytscha*. TEM of degenerating (D) and exfoliating (E) epithelial cells with associated bacteria. Arrowhead = bacterium. Bar = 3.5 μm.

Bacterial and Biofilm Morphology

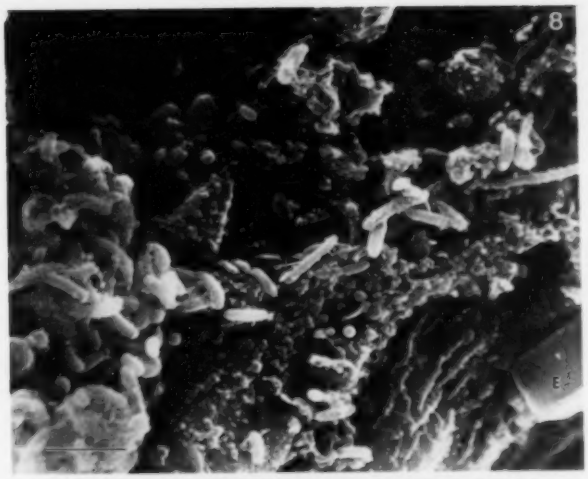
Morphologically, two types of bacteria were observed (Fig. 8 and 9). The more numerous was a short rod of 1.7–1.9 μm in length and 0.27–0.34 μm in width. The other bacteria was a serpentine rod approximately 15–25 μm in length and 0.7–1.3 μm in width. The cell walls of both were characteristic of Gram-negative bacteria. A fibrillar glycocalyx was present on the surface of both types of bacteria. Both types occurred together within microcolonies accompanied by mucus, exfoliated epithelial cells, and other debris. Additionally, large numbers of nonaggregated forms of each type occurred. These were also closely associated with epithelial surfaces.

Ultrastructurally, bacterial colonization appeared to occur along the tips of microridges. In most cases, the glycocalyx of these bacteria intermeshed with the glycocalyx of the host cells (Fig. 10). In addition to their presence on the gill surface, relatively low numbers of bacteria could be found within clear single membrane-bound vacuoles of lamellar epithelial cells.

Uniform 80- to 90-nm, round to hexagonal particles occurred sporadically and infrequently among the exfoliated debris of necrotic cells (Fig. 11 and 12). The particles had an 10- to 20-nm-wide electron-dense outer membrane, with a electron-lucent core. Within the core, many particles exhibited a 10- to 20-nm electron-dense round granule. The granules were generally epicentric within the core. These particles were never seen within intact cells.

Bacteriology

A mixed bacterial population was observed on plates at dilutions of 10^0 and 10^{-1}. The remaining dilutions contained predominantly two morphologically distinct colonies. Two colonies of nonspreading yellow pigmented bacteria (YPB) and one nonpigmented (NP) colony were selected as representatives of the CRD outbreak and were sampled from a 10^{-3} dilution plate. The isolates were slow growing, with colony size ranging from roughly 3 to 7 mm after 7 d. All three isolates grew at from 5 to 30°C but not at 37°C, grew in Cytophaga broth (CB) + 0.5% NaCl (but not in 1.0% NaCl), and failed to give a



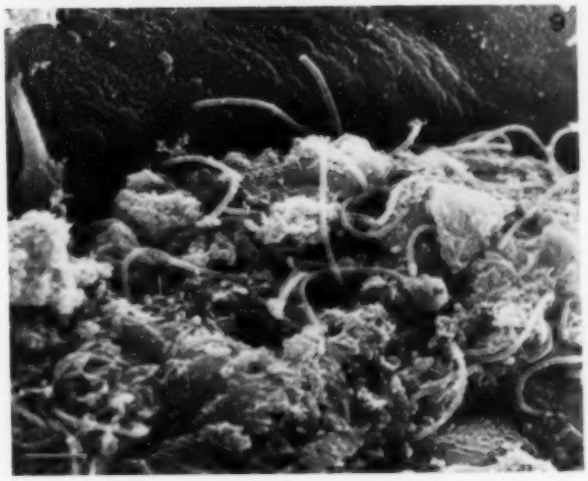
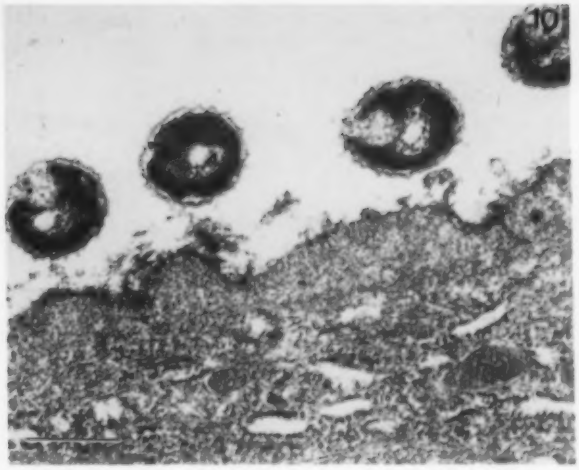
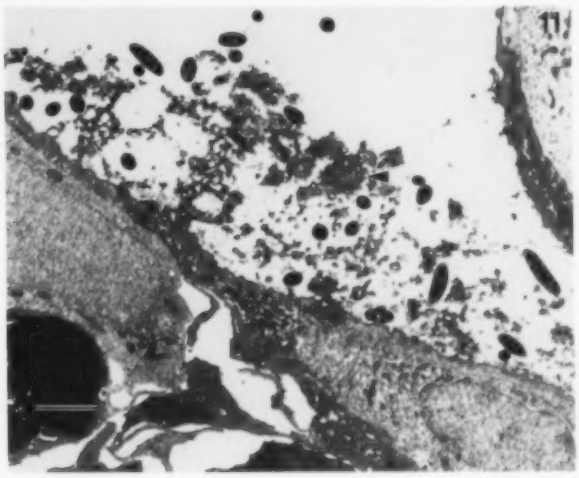


FIG. 8 and 9. *Oncorhynchus tshawytscha*. Fig. 8. SEM of short rod associated with CRD-affected branchial tissues. Arrowhead = bacterium; E = environmental contaminant. Bar = 2 μm. Fig. 9. SEM of serpentine bacteria colonizing epithelial surfaces of CRD-affected branchial tissues. Bar = 10 μm.

positive reaction in any cupule with the API 20E system, even after extended incubation periods.

The YPB isolates were Gram negative, nonmotile (flagellar or gliding) rods measuring 0.8–1.0 × 4.0–7.0 μm, oxidase and catalase positive, nonfermentative in O/F + glucose medium, and contained flexirubinlike pigments in the cell wall but did not possess flagella. Both grew aerobically on nutrient agar, trypticase soy agar (TSA), and weakly hemolytic on bovine blood agar (TSA + 5% citrated bovine blood) but did not grow on Pseudosel (Baltimore Biological Labs (BBL), Becton Dickinson Microbiology Systems, Cockeysville, MD) or on MacConkey's agar. Growth occurred under microaerophilic conditions but not under anaerobic or carboxyphilic environments using the Gas Pak system (BBL). Both YPB isolates were slightly sensitive to penicillin (10 U/disk), degraded casein, starch, and gelatin and although they failed to degrade cellulose, both colonized filter paper. Both PBS1 and PBS2 failed





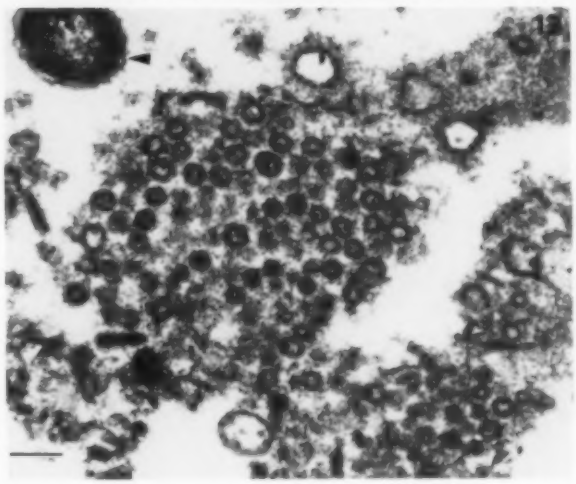


FIG. 10–12. *Oncorhynchus tshawytscha*. Fig. 10. TEM of intermeshed bacterial and cellular glycocalyxes. Bar = 0.4 μm. Fig. 11. TEM of degenerating epithelial cells, associated bacteria, and viruslike particles (arrowhead). Bar = 2 μm. Fig. 12. TEM of viruslike particles. Arrowhead = bacterium. Bar = 0.2 μm.

FIG. 2. Percent mortality (measured volumetrically) of chinook salmon alevins, naturally infected with CRD, 1 wk subsequent to chemotherapeutic treatment.

No. of alevins per tray	Control	Hyamine treated	Chloramine T treated
950	75	5	0
1493	80	2	1
1745	80	90	25

TABLE 3. Percent mortality (measured volumetrically) of chinook salmon alevins, experimentally infected with CRD, 1 wk subsequent to chemotherapeutic treatment.

No. of alevins per tray	Control	Hyamine treated	Chloramine T treated
1041	99	20	0
2631	95	95	3
2631	99	99	3
2631	95	95	1
1471	97	99	2
1471	95	99	1
1471	97	99	1
1471	99	99	2
1388	98	97	1
1388	99	99	2
1388	95	99	2
1388	96	98	1

to hydrolyze esculin or urease, to produce indole, nitrite, or H_2S from thiosulphate, and to grow on Simmons' citrate.

The NP isolate (PBS3) was a Gram-negative, motile (two to five polar to subpolar flagella) rod measuring 0.8–1.0 × 3.0–5.0 μm that was oxidase and catalase positive and nonfermentative in O/F + glucose medium. This isolate grew aerobically on nutrient agar and bovine blood agar but failed to grow on TSA, MacConkey's, or Pseudosel agar (BBL). Growth was evident on *Pseudomonas* agar F with a yellow to brown pigment evident throughout the agar after extended incubation periods (up to 14 d). PBS3 hydrolyzed gelatin and casein, failed to hydrolyze starch, esculin or cellulose, did not grow on Simmons' citrate, and failed to produce urease, indole, or H_2S from thiosulphate. Growth occurred on 1.5% CA after anaerobic and microaerophilic incubation but not under carboxyphilic conditions.

Identification

Based on the criteria of Holmes et al. (1984) and Palleroni (1984), the YPB isolates (PBS1 and PBS2) shared biochemical characteristics with *Flavobacterium* sp., while PBS3 resembled *Pseudomonas fluorescens*. Positive fluorescence was not observed on the gill surfaces of the CRD-affected fish when tested by IFAT.

Treatment

Natural infections

The results of the treatments of the three naturally infected groups of alevins are shown in Table 2. Within 3 wk posttreatment, mortality in both the Hyamine-treated and control fish approached 100%, so alevins from these groups were not ponded. After removal of the dead fish from the Chloramine-T-treated trays all remaining alevins survived and were ponded. Histologically, hyperplasia and fusion of both filaments and secondary lamellae, with absence of bacterial microcolonies, were detected 1 wk posttreatment in the Chloramine-T-treated fish; however, complete morphologic branchial recovery occurred 2–3 wk posttreatment.

TABLE 4. One-way and two-way ANOVA for tray position, week, and treatment effect of chinook salmon alevin mortality.

Factor	F ratio	df	$p < 0.01$
Tray position (two-way)	1.883	2, 18	No
Week (two-way)	0.658	2, 18	No
Tray and treatment (two-way)	0.483	4, 18	No
Week and treatment (two-way)	1.397	4, 18	No
Treatment (two-way)	1.03×10^4	2, 18	Yes
Treatment (one-way)	1.63×10^4	2, 30	Yes

TABLE 5. Student–Newman–Keuls test statistic (q) for comparing group means (30 df). p_m = number of means tested.

Comparison	Difference in means	q	p_m	$p < 0.05$
Hyamine/Chloramine T	0.96	190.1	3	Yes
Control/Chloramine T	0.95	187.7	2	Yes
Hyamine/control	0.01	2.4	2	No

Experimental infections

The results of the chemotherapeutic trials are shown in Table 3. All of the control and Hyamine-treated alevins were discarded due to the large number of mortalities and the potential risk of horizontal transmission of CRD. After removal of the dead alevins from the Chloramine-T-treated trays, the remaining alevins were ponded without incident. Histological examination of these fish 1 wk subsequent to the final treatment revealed no bacterial microcolonies; however, as was observed with the naturally infected fish, lamellar epithelial hyperplasia and fusion were still evident. Histologic examination 2–3 wk posttreatment revealed complete recovery of branchial tissue.

Statistics

The results of the ANOVA and Student–Newman–Keuls tests are presented in Tables 4 and 5. The ANOVA tests demonstrated that tray position and time of experimental infection during the CRD outbreak did not significantly alter the mean percent mortality rates ($p > 0.05$). Additionally, when these two variables were stratified according to treatment, the mean mortality rates were not significantly different ($p > 0.05$). These results permitted us to obtain and compare means from the 11 experimental trays in each of the three treatment groups because these variables did not significantly influence mortality rates. The resulting ANOVA that tested overall mortality between the three treatments yielded a significant F value ($p < 0.01$) that allowed us to conclude that the mean from at least one of the three treatment groups is significantly different from the others. The Student–Newman–Keuls test (Table 5) identified that the mean mortality rates between the Chloramine-T-treated fish and the controls, and the Chloramine-T-treated and Hyamine-treated groups are significantly different ($p < 0.01$), while the means between the Hyamine-treated and control fish are not significantly different ($p > 0.05$).

Discussion

The sequential pathology of the 1989–90 CRD epidemic strongly suggests that CRD is caused by progressive gill damage associated with the development of a bacterial biofilm. Comparisons of the morphologic nature of the gill damage, and the spatial association of lesions with a bacterial biofilm, support our assumption that the CRD outbreak investigated in 1989–90 was typical of previous outbreaks. However, this assumption is made in lieu of bacteriology results both from the 1989–90 outbreak and from those of previous years.

Several aspects are similar to previously reported gill disease of salmonids, notably BGD. Morphologically, the close spatial association of the bacterial biofilm to regions of gill damage is similar to patterns described in the commonly occurring BGD of salmonids (Speare et al. 1991b). Additionally, the specific association and interaction of the bacteria with the gill epithelial cell membrane is similar to that reported for BGD (Kudo and Kimura 1983a, 1983b; Speare et al. 1991a). Temporally, the patterns in the development of the biofilm, particularly the formation of bacterial microcolonies, with respect to the onset of gill lesions and clinical disease, also parallel the pathogenesis of BGD (Ostland et al. 1990; Speare et al. 1991b).

The bacterial isolates recovered from the 1990–91 outbreak differed in both size and morphology from the predominant bacteria observed ultrastructurally. *Flavobacterium* sp. and *P. fluorescens* have been previously reported in association with teleost branchial lesions (Miyazaki et al. 1984; Ostland et al. 1990), but their role in CRD has not been established. *Pseudomonas fluorescens* and *Flavobacterium* sp. are common components of freshwater ecosystems as well as of teleost gills (Trust 1975; Horsley 1977; Allen et al. 1983; Mudarris and Austin 1988). These isolates could therefore be normal aquatic inhabitants that secondarily colonized a previously altered gill surface, prior to the onset of clinical CRD. It is also possible that our culture conditions did not support the growth of the bacteria observed ultrastructurally. Filamentous bacteria were seen on the gills of some of the CRD fish (Fig. 9); however, the lack of immunofluorescence of CRD-affected gills when stained with anti-*F. branchophila* serum suggests that bacteria on the CRD-affected gills are not antigenically related to *F. branchophila*, a causative agent of BGD (Wakabayashi et al. 1989; Ferguson et al. 1991). Further immunofluorescent stains using antiserum prepared against the isolates recovered from this outbreak may help identify which, if any, of these isolates are involved in CRD. Alternatively, the virulence of these isolates should be determined using Harrison River strain chinook salmon alevins so that their role in the pathogenesis of CRD can be ascertained.

The pathogenesis of many infectious diseases is often multifactorial. A range of physicochemical water indices and management practices have been empirically associated with BGD outbreaks (Wood and Yasutake 1957; Snieszko 1981; Speare and Ferguson 1989). The restriction of CRD to a single hatchery strongly suggests that factors peculiar to this facility may be involved in the pathogenesis. From a production viewpoint, rearing the early stages of the CRD-sensitive Harrison River strain of chinook salmon may make this hatchery particularly susceptible to losses due to the disease. Additionally, as reported by Alderdice et al. (1987), water supplied to the Chehalis Hatchery differs from water that sustains most native chinook salmon runs in British Columbia in that the Chehalis water is slightly acidic and very soft. However, linking these differences to the observed pathogenic phenomena would be premature until specifically proven to be associated with causation. Similarly, the presence of iron particles in the affluent hatchery water and on the gills of alevins preceding and during the course of CRD warrants further investigation. The potential role of iron, entrapped within the mucous coat associated with the gill, in promoting the growth of biofilms of pathogenic bacteria has been discussed with relevance to BGD (Speare et al. 1991a). Dissolved iron salts have been shown to cause pathological changes to branchial tissue (Ashley 1970). Iron is an important factor in human oral infections, as it is an essential nutrient of both bacteria and fungi (Epstein et al. 1984). Additionally, multivalent cations have been shown to be involved with the attachment of bacteria to mucosal surfaces both in vivo and in vitro (Jones et al. 1976; Plotkin and Bemis 1984).

Characterization of the observed viruslike particles has not been possible because viral culture has not been historically successful in CRD, and there was a paucity of ultrastructurally observed particles. Respiratory pneumonia with a primary viral etiology and bacterial superinfections has been well documented in both human (Jakab 1977, 1981) and veterinary literature (Thomson 1981), and thus it is plausible that CRD may have a similar pathogenesis. In these mammalian models, however, histopathological changes consistent with viral infections are detected prior to bacterial colonization and subsequent changes. As no changes were evident either histologically or ultrastructurally, it is questionable whether these particles are involved in the pathogenesis of CRD. Therefore, until isolation, characterization, and transmission of these particles can be performed, it is impossible to draw conclusions as to their involvement with CRD at this time.

Statistical stratification of the experimentally induced treatment data has allowed us to conclude that the potential confounding variables of tray position and time during the CRD outbreak did not affect treatment results. Thus, we were able to compare 11 trays from each of the three treatments. The ANOVA showed that at least one mean was significantly different from the others. The Student–Newman–Keuls test confirmed that there was no significant difference in mortality rates between the control and Hyamine-treated trays, but Chloramine T treatment significantly reduced mortalities in comparison with the other two groups.

In summary, we feel that CRD is of a bacterial etiology based, albeit indirectly, on a number of observations and conclusions from this study. Bacteria colonized branchial tissue prior to evidence of histopathological changes and were observed to be spatially related to these diseased branchial areas. The lesions are consistent, both by light microscopy and ultrastructurally, with those of BGD. Bacteriological evidence is inconclusive; however, the bacteria in sections do not appear to be antigenically or morphologically similar to *F. branchophila*, a causative agent of BGD. Bacteria morphologically similar to those colonizing branchial tissue were associated with lesions consistent with ulcerative dermatitis. Additionally, and perhaps most importantly, the significance of the disease process can be reversed by the application of an antibacterial chemotherapeutant.

We hope that the results of this study will aid hatchery management to significantly decrease mortality at the Chehalis River Hatchery. This will be the most important result and will be determined during the next few production cycles.

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REVIEWS/SYNTHÈSES

Experimental Acidification of Little Rock Lake, Wisconsin: Chemical and Biological Changes over the pH Range 6.1 to 4.7

P.L. Brezonik[1]

Department of Civil and Mineral Engineering, University of Minnesota, Minneapolis, MN 55455, USA

J.G. Eaton

Environmental Research Laboratory, U.S. EPA, Duluth, MN 55804, USA

T.M. Frost

Trout Lake Station, Center for Limnology, University of Wisconsin, Madison, WI 53706, USA

P.J. Garrison

Wisconsin Department of Natural Resources, Madison, WI 53711, USA

T.K. Kratz

Trout Lake Station, Center for Limnology, University of Wisconsin, Madison, WI 53706, USA

C.E. Mach

Department of Civil and Mineral Engineering, University of Minnesota, Minneapolis, MN 55455, USA

J.H. McCormick

Environmental Research Laboratory, U.S. EPA, Duluth, MN 55804, USA

J.A. Perry

Department of Forest Resources, University of Minnesota, St. Paul, MN 55108, USA

W.A. Rose

U.S. Geological Survey, Madison, WI 53706, USA

C.J. Sampson

Department of Civil and Mineral Engineering, University of Minnesota, Minneapolis, MN 55455, USA

B.C.L. Shelley

Department of Plant Biology, University of Minnesota, St. Paul, MN 55108, USA

W.A. Swenson

Center for Lake Superior and Environmental Studies, University of Wisconsin, Superior, WI 54880, USA

and K.E. Webster

Wisconsin Department of Natural Resources, Madison, WI 53711, USA

Brezonik, P.L., J.G. Eaton, T.M. Frost, P.J. Garrison, T.K. Kratz, C.E. Mach, J.H. McCormick, J.A. Perry, W.A. Rose, C.J. Sampson, B.C.L. Shelley, W.A. Swenson, and K.E. Webster. 1993. Experimental acidification of Little Rock Lake, Wisconsin: chemical and biological changes over the pH range 6.1 to 4.7. Can. J. Fish. Aquat. Sci. 50: 1101–1121.

The two basins of this seepage lake were separated by a vinyl curtain in August 1984 after a year of background studies, and acidification of one basin with H_2SO_4 began at ice-out in 1985. Chemical and biological responses measured during successive 2-yr periods at pH ~5.6, 5.1, and 4.7 verified some but not all impacts predicted

[1]Corresponding author; authors listed in alphabetical order after the first author.

at the outset. Changes in major, minor, and trace ions generally agreed with predictions. Internal alkalinity generation (IAG) increased at lower pH, and sulfate reduction eliminated ~50% of added H_2SO_4. Sediment cation exchange was important in IAG and acidified surface sediments, possibly diminishing the lake's ability to counteract further H^+ inputs. Mass loss of oak leaves was reduced at pH 5.1 (birch leaves at pH 4.7). Population parameters were more sensitive than community measures for plankton. Species composition changed at each pH, especially at pH 4.7. Many changes in zooplankton and benthos were indirect responses to an algal mat that developed at lower pH or to food web interactions; these were not predicted accurately. Sensitivity of major fishes to lower pH was *Ambloplites rupestris* > *Micropterus salmoides* > *Pomoxis nigromaculatus* > *Perca flavescens*. Fish production was reduced at pH's above those resulting in population decreases.

Dans ce lac à alimentation souterraine, les deux bassins ont été séparés en août 1984 par un rideau de vinyle, après une année d'étude des données ambiantes, et l'acidification d'un bassin au H_2SO_4 a commencé dès la fonte des glaces en 1985. Les réactions chimiques et biologiques mesurées pendant des périodes successives de deux ans à des pH de ~5,6, 5,1 et 4,7 ont permis de vérifier certains des effets prévus au départ, mais pas tous. Les changements dans les ions majeurs, mineurs et traces étaient dans l'ensemble conformes aux prédictions. La production interne d'alcalinité (IAG) augmentait quand le pH baissait, et la réduction des sulfates éliminait ~50 % du H_2SO_4 ajouté. L'échange de cations dans les sédiments était important dans l'IAG et acidifiait les sédiments de surface, ce qui diminuait peut-être l'aptitude du lac à contrer des apports subséquents de H^+. La perte massive de feuilles de chêne était réduite à pH 5,1 (et à 4,7 pour les feuilles de bouleau). Les pramètres démographiques étaient plus sensibles que les mesures touchant les communautés planctoniques. La composition spécifique changeait pour chaque pH, particulièrement à 4,7. De nombreux changements observés dans le zooplancton et le benthos constituaient des réactions indirectes à la présence d'une matte algale qui se développait quand le pH baissait ou aux interactions trophiques; ces phénomènes n'ont pas été prédits correctement. La sensibilité des principales espèces de poissons à la baisse du pH peut se représenter comme suit : *Ambloplites rupestris* > *Micropterus salmoides* > *Pomoxis nigromaculatus* > *Perca flavescens*. La production de poissons était réduite lorsque le pH était plus élevé, ce qui causait des diminutions dans les effectifs.

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Experimental manipulation of whole-lake ecosystems offers several advantages over other approaches in evaluating the effects of acidification. It provides the opportunity to study indirect effects of acidification; responses that result from changes in trophic level interactions, for example, cannot be measured in the laboratory (Perry et al. 1987; Perry and Troelstrup 1988). Whereas data from lake surveys are best used to generate hypotheses, properly designed whole-lake manipulations can be used to test hypotheses and verify cause–effect relationships (Frost et al. 1988). In addition, lake manipulations provide opportunities to develop a mechanistic understanding of both chemical and biotic responses during acidification, as demonstrated by acidification experiments on drainage lakes in the Experimental Lakes Area (ELA) in western Ontario (Schindler et al. 1985).

The whole-basin experimental acidification at Little Rock Lake (LRL), an oligotrophic, low-alkalinity seepage lake in north-central Wisconsin, was designed to provide quantitative, mechanistic information on the chemical and biological effects of acidification (Watras and Frost 1989). The three-step manipulation, which was completed in the spring of 1991, complements the experiments at ELA, as well as the survey approach widely used elsewhere to assess aquatic effects of acidification. Experiments at the ELA provide valuable information with which to compare and contrast the LRL results (Schindler et al. 1992). However, the ELA lakes are different hydrologically, lie in a different geological setting, and have a different biotic community than LRL, which is representative of numerous acid-sensitive seepage lakes.

This paper describes the responses of the LRL ecosystem to experimental acidification. Preacidification data, the existence of two distinct basins (providing an opportunity for division into reference and treatment basins), and the availability of data on nearby lakes (from the University of Wisconsin's North Temperate Lakes Long-Term Ecological Research (LTER) site (Magnuson et al. 1984b) made LRL particularly attractive for this study and helped to separate pH effects and natural year-to-year variations in lake conditions.

Prior to the start of acidification, we generated a large set of predictions regarding responses of LRL to acid additions (Brezonik et al. 1985, 1986). The predictions were based on existing literature and experiments in mesoscale enclosures in LRL. The intent was to evaluate the adequacy of then-existing information, which consisted primarily of laboratory results and inferences from field surveys, as a basis for assessing the direct and indirect effects of acid loadings to lakes. The accuracy of our predictions is described in the context of the observed responses, and reasons for differences between predicted and actual responses are discussed.

The LRL experiment produced four types of findings about the ecological effects of lake acidification. First, it confirmed many hypotheses that had been generated from lake surveys but could not be proved by such approaches. Second, it elucidated *mechanisms* of responses to acidification, especially for indirect effects. Third, it provided quantitative information on chemical and biological responses, including data on the ability of the lake to *resist* acidification by internal mechanisms (internal alkalinity generation, IAG). Finally, it yielded several new findings about biotic responses to acidification.

Site Description

LRL is a two-basin glacial lake in the Northern Highland Lake District in Vilas County, Wisconsin (45°59′55″N, 89°42′15″W). The lake lies in an uninhabited, forested watershed (mixed conifers and hardwood) with highly permeable sandy soil. Public access to the site, which is in the Northern Highlands State Forest, was restricted during the study. The

TABLE 1. Average, maximum, and minimum pH of the acidified basin of LRL by treatment period.

Measure	Background, 1984–85	Treatment period (April to April)					
		pH 5.6		pH 5.1		pH 4.7	
		1985–86	1986–87	1987–88	1988–89	1989–90	1990–91
Summer avg.	6.15	5.61	5.61	5.24	5.15	4.79	4.88
Summer max.	6.33	5.80	5.74	5.39	5.33	5.02	5.04
Summer min.	6.00	5.39	5.41	5.05	5.03	4.68	4.73
Winter max.	6.13	5.50	5.63	5.22	5.27	5.12	4.90
Winter min.	5.39	5.36	5.34	4.73	5.05	4.81	4.74

FIG. 1. Bathymetric map of LRL showing location of barrier separating treatment and reference basins. Treatment basin: surface area = 9.8 ha, mean depth = 3.8 m, maximum depth = 10.3 m; reference basin: surface area = 8.1 ha, mean depth = 3.1 m, maximum depth = 6.5 m.

two basins of LRL are similar in area, but the north (treatment) basin is deeper than the south (reference) basin (Fig. 1). The south basin does not stratify, but a small hypolimnion (6% of basin volume) forms in the north basin and becomes anoxic by midsummer.

The lake is a groundwater recharge system with no surface inlets or outlets. It receives 98–100% of its water from precipitation directly on the lake surface. Most of LRL is above the regional groundwater table. Groundwater inflow occurs only in the extreme southeast corner of the lake and contributes about 2% of the lake's water inflow during relatively high precipitation years (e.g. 1984–86). During a drought in 1987–88, the water table declined below the lake level around the entire lake, and no inflow occurred. Lake levels also declined by 1.0 m during this period. Near-normal precipitation in the years following the drought was insufficient to raise the water table, and the lake continued to receive all its water directly from the atmosphere. The major water losses are evaporation (65%) and groundwater recharge (35%).

The chemistry of LRL reflects its hydrology: its water is very dilute (specific conductance ≈12 μS/cm, near that of local rain), total base cation concentrations are ~90 μeq/L, alkalinity is ~25 μeq/L, and nutrient levels are low. Major sources of elements and ions to the lake are wet and dry atmospheric deposition (Tacconi 1988; Weir 1989). Litterfall from birch, oak, maple, and conifers also provides nutrients and some ions (Perry and Troelstrup 1988). Sinks for chemical constituents include groundwater recharge, sedimentation of particulate matter, and diffusion of ions into the sediment, where reduction or sequestration occurs.

The lake supports a warmwater fish community dominated by yellow perch (*Perca flavescens*), rock bass (*Ambloplites rupestris*), and largemouth bass (*Micropterus salmoides*). A small population of black crappie (*Pomoxis nigromaculatus*) and nonbreeding individuals of a few other species are also present.

Design of Acidification Experiment

The lake was monitored during 1983–85 to establish baseline conditions. In August 1984, a vinyl curtain was installed at a natural constriction to divide the lake into treatment (north) and reference (south) basins (Fig. 1). Results through May 1985 showed the basins to be nearly identical in chemical and biological conditions (Brezonik et al. 1985, 1986). After ice-out in May 1985, a three-step acidification of the north basin began; its pH was lowered from ~6.1 to target values of 5.6 (1985–87), 5.1 (1987–89), and 4.7 (1989–91) for the 2-yr periods in parentheses. We acidified the basin by adding technical-grade sulfuric acid from a fiberglass boat, using turbulence from an outboard motor to mix the acid into the water. Measurement of pH at many epilimnetic locations showed that rapid mixing was achieved. We monitored pH at least weekly and added acid as necessary during the ice-free season to maintain the pH near target levels (Table 1). The south basin was left as a reference system.

Methods

Details of sampling and analytical procedures, as well as quality control information, are described elsewhere (Swenson et al. 1989; Brezonik et al. 1990a, 1990b; Eaton et al. 1992; Webster et al. 1992; Sampson et al. 1993). A summary of sampling and analytical methods is provided below. Water samples were collected biweekly during the ice-free season and every 5 wk under the ice at depths of 0, 4, 6, 8, and 9 m in the treatment (north) basin and 0, 4, and 6 m in the reference (south) basin. Water pumped from depth by peristaltic pump and tygon tubing was collected in prewashed polyethylene bot-

tles. Sediment porewater was sampled by Hesslein-type membrane equilibrators and analyzed by the methods described below for lake water samples (Sherman 1988).

Physical parameters were determined by standard procedures (APHA 1981) on unfiltered samples. Turbidity was measured on a Hach 2100A turbidimeter, and specific conductance was measured with a YSI model 32 meter. Color was measured by spectrophotometry at 420 nm and standardized against platinum–cobalt reference solutions. We measured pH within 4 h of sampling using a Radiometer PHM 84 or Beckman ϕ41 closed cell meter. Alkalinity (acid-neutralizing capacity, ANC) was calculated from titration to pH 3.5 by the Gran procedure.

Major cations, anions, minor and trace metals, and inorganic nutrients were measured on samples filtered in the field through in-line 0.4-μm Nuclepore® polycarbonate membrane filters. Major cations (Ca^{2+}, Mg^{2+}, K^{+}, Na^{+}) were measured by flame atomic absorption spectrophotometry (AAS); minor cations (Al, Fe, Mn) and trace metals (Cd, Cu, Pb, Zn) were analyzed by graphite furnace AAS. Anions (Cl^{-}, SO_4^{2-}, F^{-}) were determined by ion chromatography. Ammonium (NH_4^{+}) was analyzed manually by the indophenol method, and nitrate (NO_3^{-}) was measured by the automated cadmium reduction method. Total nitrogen (TN) was measured by converting all nitrogen forms to NO_3^{-} by alkaline persulfate oxidation and subsequent analysis of NO_3^{-} by the automated cadmium reduction method. Soluble reactive phosphate (SRP) was analyzed manually by the ascorbic acid method, and total phosphorus (TP) was determined similarly after persulfate digestion. Total organic carbon (TOC) was measured by digestion with persulfate and ultraviolet light using a Dohrmann IR gas analyzer, and dissolved inorganic carbon (DIC) was measured by IR analysis of gases purged from acidified samples. Chlorophyll was extracted from seston collected on 0.5-μm polycarbonate filters by 1:1 90% acetone – DMSO and quantified spectrophotometrically.

EPA quality control standards, where available, were used to check the accuracy of analyses of chemical parameters. Field and laboratory replicates for 10% of the lake samples were included in the analytical design. The precision for most chemical analyses was within 10% of the reported value; similar values were found for field precision (based on duplicate samples). Detection limits and QC data for trace metals were presented by Mach and Brezonik (1989) and Brezonik et al. (1990a); Tacconi (1988), Weir (1989), and Sampson (1992) summarized similar information for major ions and nutrients.

Information on species composition and abundance for biota was obtained by parallel field collections from the two basins (Webster et al. 1992). Filamentous benthic algae were surveyed by divers who swam transects and estimated percentage of bottom covered by filamentous masses. Pelagic zooplankton samples were collected at the same frequency as the water chemistry samples by a 30-L Schindler–Patalas trap (53-μm Nitex mesh) and preserved with 4% buffered formalin (Frost and Montz 1988). Littoral zooplankton were sampled biweekly during the ice-free season using inverted funnel traps at a 2-m (deep) site in each basin.

Benthic macroinvertebrates were collected by three types of samplers at three sites in each basin (one and two sites in 1984 and 1985, respectively). Multiplate artificial substrates (three per site) were used to collect littoral (~0.5 m) epibenthos during a 6-wk colonization period each fall (Brezonik et al. 1990b). Infauna in the sublittoral zone (4 m) were collected with a petite Ponar dredge shortly after ice-out in the spring (prior to acid additions to a new target pH). Samples were sieved through a 0.5-mm-mesh bucket and preserved with 70% ethanol. Chironomidae data were generated by pooling animals from five replicate collections at a site (three in 1984) and then randomly subsampling a total of 50 individuals for identification to genus level. Dipteran emergence rates were determined for 7- to 10-d continuous intervals throughout the ice-free season with 0.1-m^2 conical traps set 1 m below the surface at 4-m sites.

Rates of leaf litter decomposition were measured by incubating birch and oak leaves in 1-mm-mesh nylon bags at 1 m depth in each basin (Perry et al. 1987; Perry and Troelstrup 1988). Leaves were collected from trees near the shoreline just before abscission in September; litter bags were retrieved from the lake periodically over the following year. Percent ash-free dry weight (AFDW) remaining was determined from initial fresh weights and fresh-to-AFDW conversion factors. Microbial densities and levels of physiological activity were determined using SEM, X-ray analysis, direct counts, and electron transport system activity (Perry et al. 1989). Nitrogenase activity (N_2-fixation) in periphyton and benthic algae was estimated by acetylene reduction assay (Dierberg and Brezonik 1981).

Fish reproductive activity, nesting success, and survival of embryos and larval forms were determined by extensive and frequent in situ observations by divers (Swenson et al. 1989). For example, nesting success of largemouth bass was determined by direct observation of all nests in each basin on alternate days. Production rates of yellow perch larvae were estimated from twice-weekly push-net catches as the product of growth and standing crop (Swenson et al. 1989). Abundance and age structure of fish ≥age 1 were estimated from capture–recapture surveys conducted each spring and fall (Swenson et al. 1989).

Results and Discussion of Experimental Acidification

Trends in Major Ions

During the baseline period, surface water pH was similar in the two basins (Fig. 2), and both basins showed a depression during ice cover. Kratz et al. (1987) concluded that the depression was caused by a natural increase in P_{CO_2} under the ice. The winter pH depression continued in the reference basin each year, but the treatment basin displayed a more consistent winter pH during acidification, suggesting a decrease in under-ice buildup of CO_2 at lower pH. Equilibrium P_{CO_2} values computed from pH and alkalinity data and measured values of DIC were lower under the ice in the treatment basin in the years following acidification (Weir 1989; Sampson 1992). These results could be explained by slower decomposition of organic matter at lower pH, which is consistent with our findings that rates of leaf litter decomposition decreased at lower pH. These findings are corroborated by evidence of reduced rates of respiration by the microbial community attached to decomposing leaf litter (Perry et al. 1989). However, no other direct evidence is available on diminished rates of biological O_2 consumption or CO_2 production in the acidified basin.

The treatment basin pH decreased immediately upon acid addition, but further additions were necessary during the ice-free season (because of IAG) to keep the basin near the target pH. Preacidification alkalinity values were ~27 μeq/L in both basins (Fig. 2). The mean alkalinity of treatment basin surface water decreased to 15 μeq/L during the pH 5.6 stage, −4 μeq/L during the pH 5.1 stage, and −14 μeq/L during the

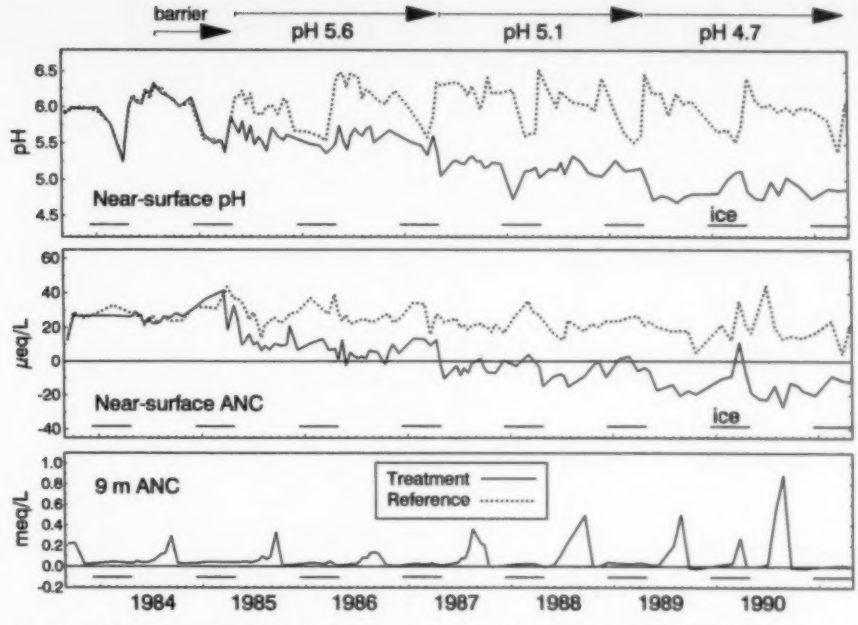

FIG. 2. pH and Gran alkalinity in surface waters (0–2 m) of the two basins and Gran alkalinity at 9 m in the LRL treatment basin over the study period. Solid line: treatment basin; dotted line: reference basin.

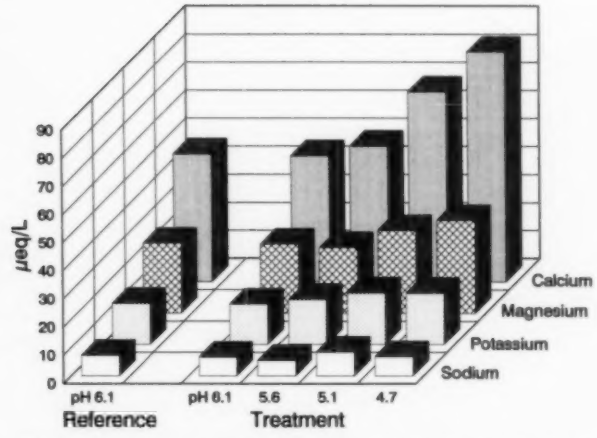

FIG. 3. Average concentrations of major cations (surface water values) in the LRL reference basin over the study period and in the treatment basin for each pH phase.

pH 4.7 stage. During the latter periods, the reference basin alkalinity declined to about 21 µeq/L because a major drought caused groundwater levels to decline around the lake, and no groundwater flowed into the reference basin after 1986.

Alkalinity in the treatment basin increased sharply in the hypolimnion during stratification (Fig. 2). Buffering processes in the sediments and hypolimnion that countered efforts to acidify the lake were apparent from vertical profiles of pH; late summer hypolimnetic pH values remained near 6.0 throughout the study in spite of acid loadings that produced epilimnetic pH values as low as 4.7. Furthermore, hypolimnetic alkalinity maxima increased during the 6 yr of acid additions (Fig. 2), most likely as a result of higher sulfate concentrations, which led to increased microbial sulfate reduction rates in the anoxic hypolimnion. For example, late summer values at 9 m averaged 250 µeq/L in 1983 and 1984 (before acidification began), but values were ~500 µeq/L by 1988 and 1989 and reached 880 µeq/L in 1990.

Ca^{2+} increased only slightly (7%) in the treatment basin at pH 5.6 but substantially (51%) at pH 5.1 and even more (82%) at pH 4.7 (Fig. 3). The reference basin [Ca^{2+}] remained below 50 µeq/L throughout the study. Concentrations of K^+ increased slightly at each target pH, resulting in about a 27% increase at pH 4.7 compared with initial conditions. Mg^{2+} increased by about 20 and 35% of initial values at pH 5.1 and 4.7, but no differences between the basins could be discerned at pH 5.6 (Fig. 3). Predicted increases in Ca^{2+} and Mg^{2+} were close to those we observed. For example, based on results of mesocosm-scale enclosure experiments conducted in the lake in 1984 (Perry 1987), we predicted increases of 75–100% in Ca^{2+} and 20–30% in Mg^{2+} at pH 5.0 and observed increases of 50 and 20%, respectively, at pH 5.1. However, our prediction that K^+ would not increase was incorrect.

The increased base cation concentrations in the treatment basin water column can be accounted for by loss of exchangeable base cations from the surficial sediments (Sampson et al. 1992). Based on samples collected in 1988 (pH 5.1 phase), we found that the upper 2 cm of reference basin sediments contained 24.6 meq exchangeable cations/100 g and the upper 2 cm of treatment basin sediments contained 11.9 meq/100 g. At least some of the difference between the basins can be attributed to partial loss of cations to the lake water during the first 3 yr of acid loadings. If the above amounts were released into the treatment basin water column, they would

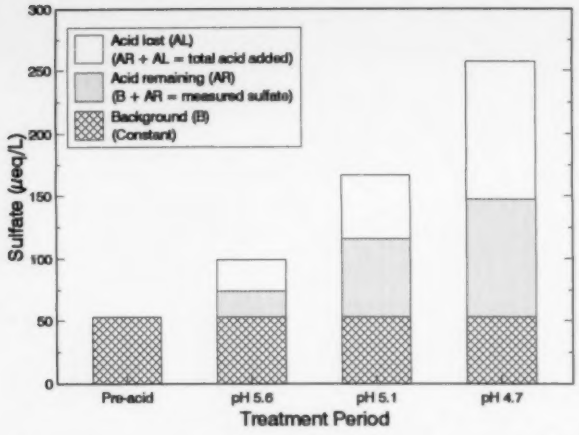

FIG. 4. Average concentrations of sulfate in surface waters of the LRL treatment basin for preacidification and three acid-treatment periods. Note: the reference basin remained at the background level throughout the study.

raise the total cation content by 60 and 29 μeq/L, respectively. The total cation concentration remained constant in the reference basin but increased by 50 μeq/L as the treatment basin pH decreased from 6.1 to 4.7. Exchange of cations in just the top few centimetres of sediments with H^+ from the water thus can account for the water column increase.

The increase in base cations, especially Ca^{2+}, has important biological and chemical implications. Biologically, the increase helps ameliorate the toxicity of increased aqueous concentrations of H^+, Al, and (possibly) heavy metals to sensitive organisms (Brown 1981; Mount et al. 1988). Chemically, the increase reflects IAG by sediment ion exchange ($2H^+_{aq}$ + Ca-Sed → Ca^{2+}_{aq} + $2H^+$-Sed). Although this counteracted water-column acidification, it came at the cost of acidifying the near-surface sediments. Evidence for this is provided by porewater pH profiles (described later), as well as measurements of porewater and solid-phase Ca levels (Weir 1989; Sampson et al. 1993).

Because sulfuric acid was used for acidification, an increase in treatment basin [SO_4^{2-}] was expected, but because of sulfate reduction in the sediments and hypolimnion, measured values were lower than calculated from the amounts of acid added (Fig. 4). Prior to acidification, the average sulfate concentration in both basins was 53 μeq/L (2.5 mg/L). After 6 yr of acid additions, the treatment basin [SO_4^{2-}] increased to ~147 μeq/L at pH 4.7. If all the SO_4^{2-} added as H_2SO_4 had remained in the water column of the treatment basin, SO^{2-}_4 concentrations at the end of the treatment period would have been 257 μeq/L (this assumes no consumption by in-lake processes and no loss by water outflow). Therefore, ~54% of the SO_4^{2-} added to the treatment basin as H_2SO_4 remained in the water column at the end of the study. Some loss can be attributed to groundwater recharge, but some was lost internally by sulfate reduction. The reference basin [SO_4^{2-}] remained near preacidification levels through 1990, and neither basin showed any noticeable seasonal variations in sulfate. Our predictions for [SO_4^{2-}] resulting from acidification (83, 125, and 187 μeq/L at pH 5.5, 5.0, and 4.5, respectively) were slightly higher than observed, but the final target pH values and actual values achieved were not as low as those on which the predictions were based.

Internal Alkalinity Generation (IAG)

We studied the internal processes regulating alkalinity and pH in LRL by mesoscale enclosure experiments (Perry 1987), measurements of porewater chemistry (Sherman 1988; Sherman et al., in review), ion budget calculations (Tacconi 1988; Weir 1989), reactor models (Baker and Brezonik 1988), and analysis of chemical trends in the lake. The major IAG processes in the lake are microbial sulfate reduction and cation input to the water column by sediment ion exchange. Nitrate reduction also consumes H^+, but net retention of NH_4^+ in the lake has the opposite effect, and these two processes approximately balance each other (Brezonik et al. 1987).

IAG in Little Rock Lake takes place primarily in the sediments. Sulfate reduction below the oxic/anoxic interface causes sulfate to diffuse from the water column into the sediments. Although reoxidation of reduced sulfur compounds occurs above the oxic/anoxic interface, the sediments are *net* sinks for sulfur, which is sequestered as both organic S and mineral forms (Baker et al. 1989, 1992). Increased cation concentrations in the lake water could result from three processes: (i) exchange of H^+ for base cations on sediment exchange sites, (ii) release of cations during mineral weathering, and (iii) liberation of cations during decomposition of organic matter. As described earlier, the ion-exchange capacity of surficial sediments can account for the increased base cation concentrations in the treatment basin water column and thus for the associated IAG. X-ray analysis revealed the presence of clay minerals in LRL sediment (Weir 1989), but the importance of aluminosilicate weathering as a source of cations in the lake cannot be estimated from existing information. Cation release from decomposing seston accounts for ~10–15% of the major cation budgets for the LRL water column (Baker et al. 1988), but cations released this way mostly represent the recycling of cations removed from the epilimnion during planktonic growth. Some Ca^{2+} is released from incoming leaf litter, but the amounts are small compared with inputs from atmospheric deposition (B. Shelley, R. Hoisve, and P. Brezonik, unpublished data). Sediment ion-exchange thus was probably the major cation-related IAG mechanism during treatment basin acidification.

Porewater profiles for SO_4^{2-}, Ca^{2+}, pH, and alkalinity at a 5-m site in the treatment basin (Fig. 5) illustrate the IAG processes described above and show that sediment conditions changed during acidification. Much higher pH and alkalinity values occur below the sediment/water interface, and the changes in Ca^{2+} and SO_4^{2-} below the interface are as expected from the IAG processes. Large changes in all four parameters occur at about the same depth for a given date and site. However, the changes occurred about 10 cm below the interface in 1990 but at or just below the interface in earlier years. Corresponding profiles for the reference basin in 1990 did not show a depression, and it is apparent that acidification of the upper sediment of the treatment basin occurred during the latter half of treatment. Neutralization of acidified sediments (replacement of cations lost during acidification) will be a part of the lake's recovery process, and the time required to achieve this will need to be considered in predicting the rate of LRL recovery (Sampson et al. 1993).

Annual budgets for ions involved in IAG were computed by Tacconi (1988) and Weir (1989) for the first phases of the study;

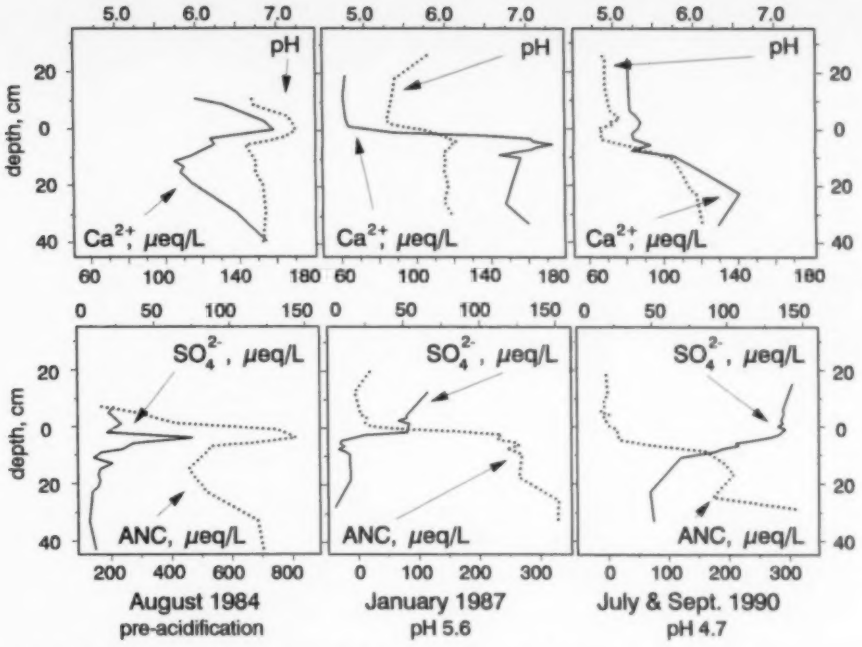

FIG. 5. Vertical profiles of pH, Gran alkalinity, and SO_4^{2-} and Ca^{2+} concentrations in sediment porewater and overlying water column at the 5-m site in the LRL treatment basin for three time periods representing the approximate beginning, midpoint, and end of the acidification period.

water budgets for the last three treatment years are still incomplete, and ions budgets thus are not available. In brief, the available budgets showed that areal rates of sulfate reduction and IAG were nearly the same in the two basins in the pretreatment year, but rates of these processes doubled in the treatment basin (from 27 to 53 $meq \cdot m^{-2} \cdot yr^{-1}$ (sulfate) and from 46 to 89 $meq \cdot m^{-2} \cdot yr^{-1}$ (IAG)) during the first three treatment years, probably because of higher water column $[SO_4^{2-}]$ (Sampson et al. 1993). Only 33% of the added acid resulted in decreased lake alkalinity (Weir 1989). The rest was lost by out-seepage or neutralized by IAG processes, of which sulfate reduction accounted for about 60%.

Trace and Minor Metals

Concentrations of dissolved Al, Mn, Fe, Zn, Cd, and Pb increased (the last two only slightly) at lower pH in the treatment basin during the experiment (Fig. 6); dissolved Cu did not change, averaging 0.2 μg/L in both basins for the duration of the study. Only traces of heavy metals were found in the sulfuric acid used to acidify the lake, and they could not explain the increases we measured in the treatment basin. Except for Pb, measured changes in dissolved metal ions generally agreed with results from mesoscale acidification experiments with enclosures in LRL and laboratory experiments in which LRL sediments were extracted at different pH values (Downing, 1986; Brezonik et al. 1990a; Mach 1992). We correctly predicted the increases in Al, Mn, Cd, and Zn and the lack of increase in Cu, but we predicted that Fe would not increase because sulfide would precipitate Fe solubilized at lower pH, and this was incorrect.

Dissolved Fe and Mn concentrations increased almost continuously in the treatment basin from the start of acid additions. The higher concentrations at lower pH are probably due to increased solubility of amorphous ferric hydroxide and manganite (MnOOH); computed equilibrium values for solubility of these phases agree closely with measured concentrations (Mach 1992). The decrease in dissolved Fe at pH 4.7 may have been caused by coprecipitation with aquatic humus (Mach 1992); color and TOC values also declined at this pH.

Dissolved Al was not expected to increase to high levels in LRL at lower pH because the lake receives no surface runoff. Based on acidification experiments in enclosures during 1984 and 1985 (Mach and Brezonik 1989), we predicted modest Al increases during acidification: 9–20 μg/L at pH 5.0; 20–45 μg/L at pH 4.5 (the target pH values at the time the predictions were made). Dissolved Al observed at pH 5.1 (surface water average = 28 μg/L) was higher than we predicted for pH 5.0; the average at pH 4.7 (38 μg/L) was near the upper end of our prediction for pH 4.5. These values are much lower than those found in acidic drainage lakes of the northeastern United States (hundreds of micrograms per litre; Schafran and Driscoll 1987) and lower than values cited as causing fish toxicity (~200 μg/L; e.g., Campbell et al. 1985). Nonetheless, in laboratory bioassays with simulated LRL water (Eaton et al. 1992; McCormick and Jensen 1992), increased mortality of largemouth bass, yellow perch, and rock bass larvae occurred at Al levels near those observed in LRL. For example, survival of yellow perch larvae to 7 d post-hatch at pH 5.0 decreased from ~75% at 10–45 μg total Al/L (6–25 μg inorganic monomeric Al/L) to 43% survival at 85 μg total Al/L (55 μg inorganic monomeric Al/L).

Water column increases in Cd and Zn and the lack of increase in Cu at lower pH conform to results of extraction measurements to determine how metals in the sediments are partitioned among major chemical forms (Downing 1986). Sedimentary

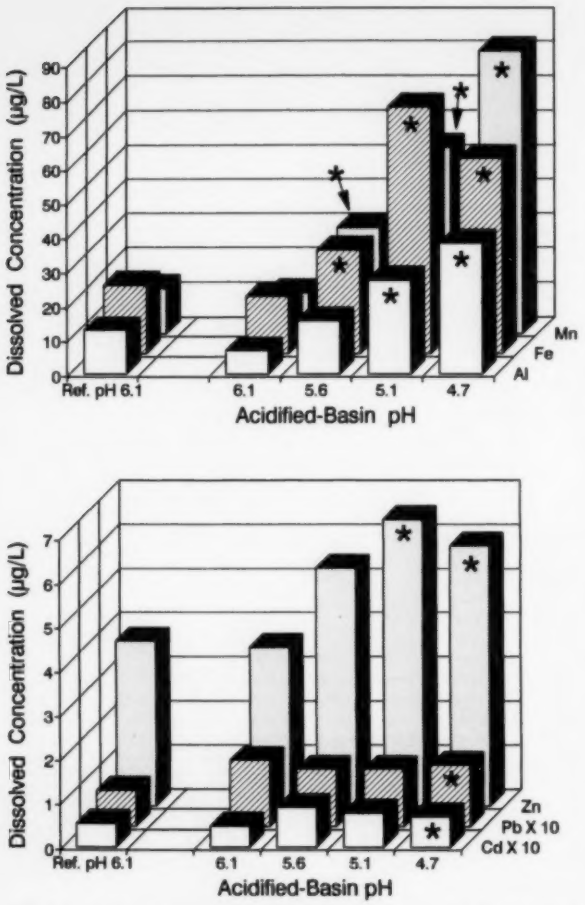

FIG. 6. Average concentrations of dissolved minor and trace metals (epilimnetic values) in the LRL reference basin over the study period and in the treatment basin for each pH phase. An asterisk on a treatment basin bar indicates a significant difference from the reference basin at at least the 95% confidence level (based on Wilcoxon's two-sample test). Dissolved Cu (not shown) remained similar in both basins down to pH 4.7.

Cd and Zn are associated primarily with the ion-exchangeable fraction and thus are more sensitive to pH changes than Cu, which is associated primarily with the less-labile organic/sulfide fraction in the sediments. Elevated levels of Cd at reduced pH have not been reported in previous acidification experiments (Hall et al. 1980; Schindler et al. 1980a, 1980b), but we found increased dissolved Cd levels at pH 5.1 and 4.7 in LRL and at pH 4.5 in mesoscale enclosures in LRL (Mach and Brezonik 1989; Brezonik et al. 1990a). The lower pH required to induce elevated dissolved Cd levels in the enclosures probably is an artifact of the short (3 mo) duration of these studies; indeed, dissolved Cd levels in the treatment basin of LRL were not elevated above reference basin levels until year 2 at pH 5.1. Elevated lake water Cd levels are of interest, since this metal has a high inherent toxicity to aquatic biota (Kaiser 1980) and elevated levels have been reported in fish of low-alkalinity lakes (Wiener 1983, 1987).

Dissolved Pb levels were similar in the two basins until pH 4.7 when acidified basin levels were ~80 ng/L higher than reference basin levels (Mach 1992). This result was unexpected. In our enclosure experiments, Pb was not detected either in the controls (pH 6) or in treatment enclosures down to pH 4.5 (Mach and Brezonik 1989). Also, in laboratory experiments with LRL sediments, Pb did not desorb until pH < 4 (Downing 1986; Brezonik et al. 1990a). The difference between the whole-lake and enclosure results may be explained by the short duration (3 mo) of the latter experiments (compared with 2-yr treatment periods for the lake). The difference between the whole-lake results and the laboratory desorption study suggests that the higher levels in the lake at pH 4.7 may be from atmospheric deposition to the lake surface and reduced scavenging from the water column at low pH.

Bioaccumulation

The effects of pH on Cd bioaccumulation varied widely depending on the position of organisms in the LRL food web (King et al. 1992). Mean Cd concentrations in periphyton in the treatment basin (pH 4.7) and reference basin were 1.8 and 7.5 µg/g dry wt, respectively. The trend of these results agrees with predictions from a surface complexation model (e.g., Xue et al. 1988) and H^+ competition theory. The much higher aqueous concentration of H^+ at pH 4.7 apparently caused more metal-binding sites on algal surfaces to become protonated and decreased the binding by other cations such as Ca^{2+} and Cd^{2+}. In contrast, Newsom (1987) found that Cd levels in bacteria on leaf litter in the treatment basin at pH 5.6 were elevated compared with reference basin conditions.

Similarly, mean Cd concentrations in zooplankton were 2.4 times higher in the treatment basin at pH 5.1 than in the reference basin, in spite of apparently lower Cd concentrations in the food supply (algae) of treatment basin zooplankton. Differences in species composition between the basins could explain the zooplankton trends. The zooplankton samples analyzed for Cd from the treatment basin were dominated by *Diaptomus minutus* and *Asplanchna* sp., while the reference basin samples were dominated by *Holopedium gibberum, Diaphanosoma* sp., and *D. minutus*. In this regard, Yan et al. (1990) reported interspecies variations and seasonal variations of Cd within *H. gibberum* that were larger than the differences we observed between LRL basins.

Mean Cd concentrations in whole yellow perch (stomach removed), muscle tissue, skin and scales, gill tissue, and gut contents all were lower in the acidified basin. The greatest between-basin difference was observed for gill tissue: 0.085 ± 0.037 µg/g wet wt for the acidified basin and 0.35 ± 0.22 µg/g wet wt for the reference basin. The difference suggests that H^+ competition may control Cd levels in gill tissue (and possibly in the whole fish). However, other mechanisms of metal uptake and removal (i.e., ingestion and excretion) probably are involved as well. The lower levels of Cd in yellow perch from the acidified basin are the opposite of the trend reported by Wiener (1983) based on lake survey data. He found increasing Cd and Pb concentrations in sunfish with decreasing pH in Wisconsin lakes. At present, we have no reasonable explanation for these differences.

Nutrients

No differences in seasonal patterns of TP or SRP were found in the surface waters of the treatment and reference basins during the first two treatment periods (pH 5.6 and 5.1), which agrees with our predictions for these pH values. At pH 4.7, there still was no difference between basins in the annual and

summer mean values and spring maximum for TP, but we detected a slight increase in the summer mean of SRP in the treatment basin (Sampson et al. 1993). Based on the oligotrophication hypothesis, we had predicted that a small decrease in TP and SRP would occur at pH 4.5. The lack of a decrease in treatment basin TP and SRP is interesting in view of our findings that LRL sediments bind phosphate much more strongly at lower pH (Detenbeck and Brezonik 1990a, 1990b). This implies that pH-insensitive factors, most likely the balance between biotic uptake and release, control water column P levels.

No differences in near-surface TN concentrations were observed at pH 5.5 and year 1 at pH 5.1. During year 2 at pH 5.1 and both years at pH 4.7, TN in the near-surface waters of the treatment basin was consistently lower than that in the reference basin; summer average concentrations were 70–150 μg/L lower in the treatment basin. We correctly predicted the decrease in TN concentrations at pH 4.5, but we also predicted that inorganic N concentrations would decrease, and that was not observed.

Seasonal patterns of nitrate and ammonium in the surface waters of the two basins were similar over the study period and provide only slight evidence of disruption in the processes that produce and consume these ions at pH as low as 4.7. Nitrate exhibited no vertical trends in the water column at any time of year, but concentrations of $NH_4^+ > 1000$ μg/L occurred in the treatment basin hypolimnion during summer. Concentrations of NH_4^+ and NO_3^- peaked under the ice during late winter each year and declined rapidly after ice-out. The peaks in NH_4^+ under the ice probably were caused by lower rates of planktonic uptake. These high concentrations allowed growth of nitrifying bacteria under the ice, but plankton assimilation kept [NH_4^+] too low for nitrifiers to compete during the ice-free season. Summer mean concentrations of NO_3^- and NH_4^+ were slightly higher in the treatment basin at pH 4.7, but both basins were below 10 μg N/L (Sampson 1992). Coupled with the decreased TN and higher water clarity at pH 4.7 (see below), these results suggest that planktonic uptake of inorganic N was slightly diminished at pH 4.7 because of lower algal abundance. It must be noted, however, that we did not detect a difference in chlorophyll levels between the two basins, even at pH 4.7.

Lower nitrate concentrations in the treatment basin than in the reference basin during the first winter at pH 4.7 suggested that nitrification was partly suppressed under the ice, but data for the second winter did not corroborate this (Sampson et al. 1993). In contrast, Rudd et al. (1988) observed disruption of nitrification at pH 5.4–5.7 in Lakes 223 and 302S at the ELA; large increases in [NH_4^+] under the ice were coupled with lower [NO_3^-] peaks. Rudd et al. (1988) concluded that nitrifying bacteria could not adapt to the moderately acidic conditions of pH 5.4–5.7, but our data indicate that this conclusion should not be extended to all lakes. The natural pH depression during ice-cover in LRL, which produces pH values as low as 5.4, may have allowed the development of a nitrifying flora with a broader tolerance to low pH.

Annual average concentrations and seasonal patterns of dissolved reactive silica were not affected at pH 5.6 and 5.1, but reference basin concentrations were consistently lower than values in the treatment basin at pH 4.7. Based on published reports of decreased diatom abundance in lakes at pH < 5.0, we had predicted that this would occur, but phytoplankton data for the pH 4.7 period are not available to verify or disprove this explanation.

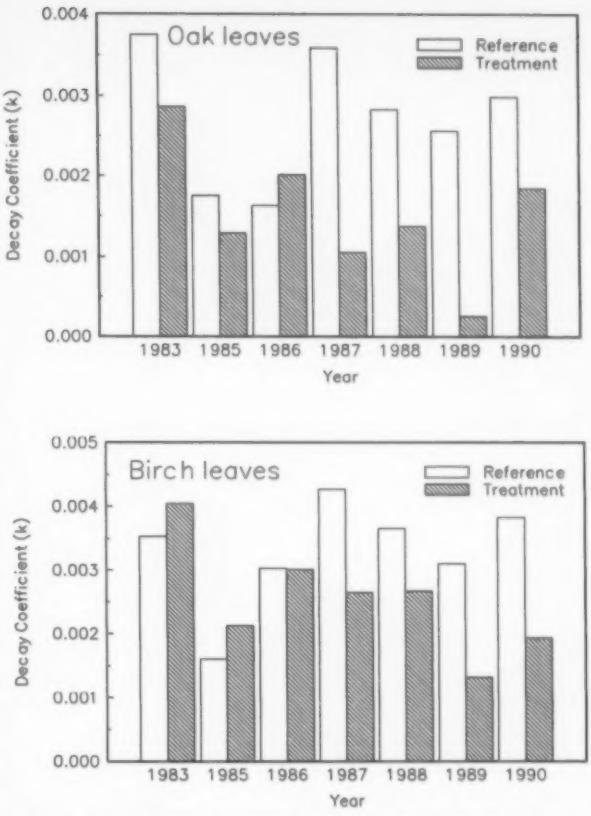

FIG. 7. Rates of litter decomposition for northern red oak and paper birch leaves in the LRL reference and treatment basins at the three treatment pH values plotted as first-order rate coefficient, k, from the slope of the regression of $\ln(wt_t/wt_0)$ versus time in days.

Leaf litter decomposition, a microbial process involved in nutrient cycling, was affected by acidification. The response varied between the two species studied; decomposition rates of oak leaves (a low quality substrate) were strongly affected at pH 5.1 (Fig. 7), but only a slight decrease was noted in birch leaf decomposition at this pH. Decomposition of both species was significantly lower at pH 4.7 (83% reduction for oak; 63% for birch) than in the reference basin and nearby LTER basins. These changes exceeded our predictions that decomposition rates would be reduced by 25% at pH 4.5. Small-scale processes related to leaf decomposition also were affected. For example, bacterial colonization rates and bacterial activity were lower at pH 5.1 and possibly at pH 5.6 than under reference conditions.

Transparency-Related Parameters

TOC decreased to about two-thirds its initial value in the treatment basin after acidification to pH 4.7, and color decreased to less than half its initial value (Table 2). Average TOC and color concentrations (surface water) of the two basins were significantly different at pH 4.7 but not at higher pH values. Schindler and Turner (1982) found no change in DOC concentration with acidification of Lake 223 to pH 5.4, but they noted decreases in DIC and suspended C. The decrease in color at pH 4.7 agrees qualitatively with survey data on low-alkalin-

TABLE 2. Mean values of transparency-related parameters for the LRL basins. Values are surface water (except transparency) averages over ice-free periods.

		Treatment period			
	Basin	Preacid	pH 5.6	pH 5.1	pH 4.7
Color (PCU)	Treatment	12.2	12.6	10.3	6.0
	Reference	13.5	16.0	12.5	10.8
TOC (mg/L)	Treatment	2.49	3.24	3.29	1.99
	Reference	2.90	3.04	3.68	3.65
Turbidity (NTU)	Treatment	0.45	0.45	0.65	0.95
	Reference	0.55	0.50	0.55	0.80
Chl *a* (mg/m^3)	Treatment	2.7	2.1	3.6	3.9
	Reference	2.0	2.3	2.9	3.7
Secchi transparency (m)	Treatment	4.9	5.3	4.6	4.7
	Reference	5.1	4.5	4.3	4.5

ity lakes in many regions. No consistent trend in Secchi transparency occurred in the treatment basin during acidification (Table 2), although water changed in hue (Watras and Baker 1988) and appeared more transparent to some observers. The highest transparency occurred at pH 5.6, when turbidity and chlorophyll levels were lowest. Mean transparency at pH 4.7 was nearly the same as that at pH 6.1. Although color was lowest at this pH, chlorophyll reached its highest levels in the treatment basin during this period. period. Apparently, the interplay among the light-scattering and light-absorbing constituents combined to yield no simple trend in Secchi transparency.

Primary Producer Communities

No significant differences between the basins could be discerned in temporal trends of chlorophyll *a* levels, even at pH 4.7 (Table 2). Interbasin differences were found in phytoplankton species composition at pH 5.6 and 6.1. In general, chrysophyceans decreased and dinoflagellates increased over this pH range (Schindler et al. 1992), and *Anabaena*, a common blue-green in the lake before acidification, disappeared from the north basin by pH 5.1 (Webster et al. 1992).

Extensive growths of filamentous benthic algae were the most visual response to acidification and one of the most rapid responses. Thick clouds of filamentous algae were observed early in the first summer of acid additions (target pH 5.6). They reached a maximum areal extent in year 1 at pH 5.1, covering most of the littoral zone (0–2 m) and portions of the basin to 6 m depth (Webster et al. 1992). The filamentous growths were mostly green algae (primarily *Mougeotia* and *Oedogonium*) that peaked in coverage by mid- to late summer each year. Although small, isolated clumps of filamentous algae were observed in the treatment basin before acidification and in the reference basin during the treatment period, they never approached the abundance or areal coverage observed under acidic conditions.

We did not regularly monitor the species composition of littoral periphyton in LRL (excluding the filamentous growths described above), but two sets of observations suggest that changes occurred. First, microscopic examination of periphyton samples from the two basins in 1988 (year 2 at pH 5.1) showed an absence of blue-green species in treatment basin samples (A. Klemer, University of Minnesota, Duluth, MN, personal communication). Second, measurement of N_2 fixation by the acetylene reduction assay on samples of periphyton and benthic algae in summer of 1990 showed that nitrogenase activity was virtually absent in the treatment basin (n = 18; one trace response) but was common in the reference basin (Sampson 1992).

Zooplankton

Responses varied markedly among zooplankton taxa and between species-level and community-level parameters. Some responses were consistent with previous studies and with our predictions, but there were several surprising changes with acidification. Figure 8 summarizes trends in the average (ice-free season) biomass for 12 common zooplankton taxa (four each among the rotifers, cladocerans, and copepods) over the experiment and illustrates the range of responses of LRL zooplankton to acidification.

Among rotifers, *Keratella taurocephala* biomass increased during year 1 at pH 5.6, but this early change was dwarfed by subsequent increases at lower pH values. In contrast, populations of *Keratella cochlearis*, *Kellicottia longispina*, and *Asplanchna priodonta* decreased at varying times during the course of the experiment. Among cladocerans, *Daphnia catawba* biomass increased greatly during year 2 at pH 5.6 and maintained high levels during the remainder of the study. *Holopedium gibberum* biomass declined as *D. catawba* increased and was nearly extirpated at lower pH levels. *Daphnia dubia* and *Diaphanosoma birgei* declined at pH 5.1 and 4.7. Copepods, which account for most of the zooplankton biomass in LRL, generally repsonded to acidification later than rotifers and cladocerans. The biomass of *Diacyclops thomasi* declined at pH 5.1, but decreases for *D. minutus* and *Mesocyclops edax* were noted only at pH 4.7. A subtle increase in biomass of *Tropocyclops prasinus* at pH 5.1 was followed by a much larger increase at pH 4.7.

With the exception of *K. cochlearis* (see below), information at a finer scale — population abundances on individual sampling dates for 34 dominant taxa in LRL (Table 3) — yielded similar patterns to that for annual average biomass. Both positive and negative shifts were evident, even at pH 5.6, and the number of responding taxa increased with each subsequent lowering of pH. At pH 5.6, populations of seven taxa declined and two (*D. catawba* and *K. taurocephala*) increased markedly. At pH 5.1, 14 taxa declined and four increased. At pH 4.7, only six species remained at levels comparable with the preacidification period, while 20 taxa declined and eight increased. No change was evident in annual mean biomas of *K. cochlearis* at pH 5.6 (Fig. 8), but population assessments based on biweekly samples indicated significant declines for this species (Table 3; and Gonzalez et al. 1990).

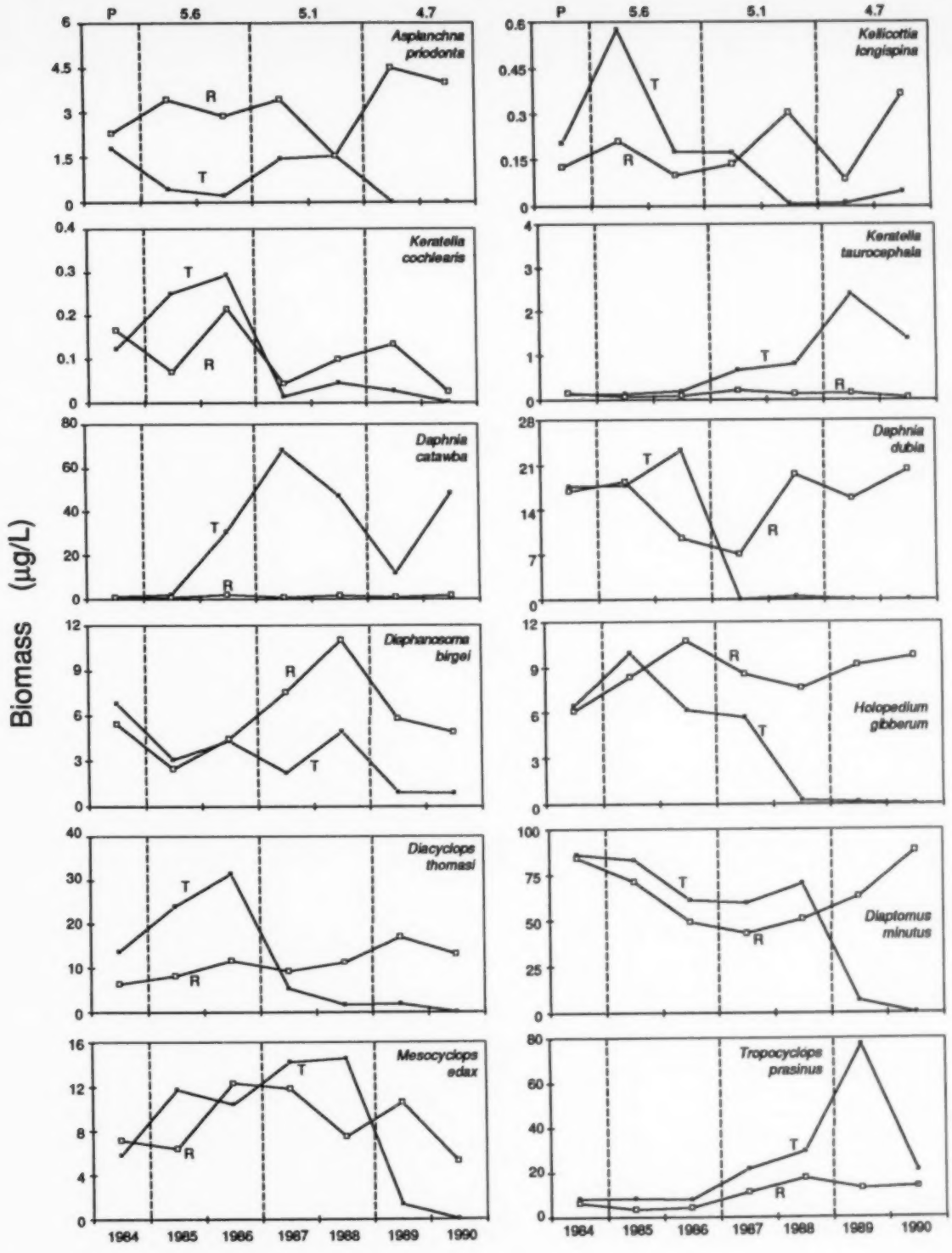

FIG. 8. Mean annual biomass of representative rotifer, cladoceran, and copepod species during ice-free periods in the LRL treatment and reference basins.

TABLE 3. Treatment basin responses to acidification for population abundances of dominant zooplankton taxa in LRL. All taxa listed that occurred in more than 3 yr in either basin and accounted for at least 0.02% of the maximum annual mean zooplankton biomass (either basin). Assessment of increase or decrease based upon comparison of paired population levels in the two basins on at least 30 dates during each acidification period, combined with information from the baseline period (1984–85). 0 = no obvious change; − = decline; + = increase.

Taxon	Target pH		
	5.6	5.1	4.7
Copepods			
Diacyclops thomasi	0	−	−
Mesocyclops edax	0	0	−
Tropocyclops prasinus	0	+	+
Diaptomus minutus	0	0	−
Epischura lacustris	−	−	−
Cladocerans			
Bosmina and *Eubosmina*	0	0	0
Daphnia dubia	0	−	−
D. parvula	0	0	0
D. catawba	+	+	+
Diaphanosoma birgei	0	−	−
Holopedium gibberum	−	−	−
Leptodora kindtii	−	−	−
Rotifers			
Ascomorpha spp.	0	0	0
Asplanchna priodonta	−	0	−
Collotheca mutabilis	−	−	−
Conochiloides sp.	0	0	0
Conochilus sp.	0	−	−
Gastropus hyptopus	0	0	−
G. stylifer	0	0	+
Kellicottia bostoniensis	0	0	−
K. longispina	0	−	−
Keratella cochlearis	−	−	−
K. crassa	0	+	−
K. hiemalis	0	0	−
K. taurocephala	+	+	+
Lecane sp.	0	0	0
Polyarthra dolichoptera	−	−	−
P. remata	0	0	+
P. vulgaris	0	0	+
Synchaeta sp.	0	0	+
Trichocerca birostris	0	−	−
T. cylindrica	0	−	−
T. multicrinis	0	0	0
Insects			
Chaoborus spp.	0	+	+

Aggregated community-level parameters, such as total biomass in the three major groups of zooplankton, were less sensitive to acidification than were individual populations (Fig. 9). On this basis, only the cladocerans gave a clear indication of a response at pH 5.6 and 5.1, but cladoceran biomasses were again similar in the two basins at pH 4.7. Total rotifer biomass may have increased during year 2 at pH 4.7, but the differences are not large compared with the variability in rotifer biomass in the reference basin during the study. A shift in total copepod biomass was evident only at pH 4.7. Finally, the total biomass of the zooplankton community remained quite constant in the treatment basin until pH 4.7 (Fig. 9).

The minimal responses that occurred in parameters that represent the aggregated behavior of several component species indicate that substantial compensatory processes are operating to control zooplankton biomass during all but the most stressful pH conditions in LRL. Declines in species responding to decreased pH generally were matched by increases in other taxa.

The pH responses of some zooplankton taxa match those reported in other studies. For example, a decline of *K. cochlearis* and increase of *K. taurocephala* with decreasing pH has been observed frequently (MacIsaac et al. 1987). Similarly, the abundance of *D. catawba* increased in another experimentally acidified lake (Malley et al. 1982). However, there also are notable differences between responses in LRL and those reported previously. In particular, *H. gibberum*, *D. birgei*, *M. edax*, and *D. minutus*, which are common dominants in acidic lakes (Brett 1989), declined substantially at lower pH in LRL.

Responses of populations of *Bosmina* spp. and *Epischura lacustris*, as well as the response of total zooplankton biomass, were consistent with our preacidification predictions (Brezonik et al. 1986). In some cases, predicted changes took place earlier than we anticipated (e.g., increases in *K. taurocephala* and declines in *Leptodora kindtii*). In several instances, our predictions were simply wrong. We predicted that *D. thomasi* would become a dominant component of the zooplankton community at pH 4.7; instead it declined to near zero. In contrast, *T. prasinus*, *Polyarthra remata*, and *P. vulgaris* were expected to decrease in abundance, but all responded positively to lower pH.

Our results suggest that several of the observed changes in zooplankton populations were driven by indirect mechanisms rather than simple direct responses to acid conditions (Webster et al. 1992). For several taxa, e.g., *K. longispina* and *H. gibberum*, biomass declined much more in year 2 of a pH treatment than in year 1. Such delayed responses would be unlikely if the organism were responding directly to physiological stress. The increased abundance of some taxa of littoral zooplankton at pH 5.1 (Table 3) probably was induced by the increased coverage of the habitat by the filamentous algal mat. Small-scale experiments (Gonzalez 1992) have shown that population changes by two rotifer species in LRL were not directly caused by pH shifts but rather by changes in the lake's food web. Our difficulties in predicting the responses of particular taxa could have occurred because the species were responding to differences in food webs rather than directly to acid conditions. If the response of a species in a previous study had been driven by an acid-related change in another taxon, a similar change might not have occurred in LRL if the responding taxon was not present.

Benthos

The benthos invertebrate community of LRL is characterized by the presence of acid-tolerant taxa. Even within groups considered sensitive to acidity, such as amphipods and mayflies, taxa found in LRL tend to be among the most acid tolerant. The LRL amphipod *Crangonyx gracilis*, for example, is more typical of acidic lakes, in contrast with sensitive genera of amphipods, such as *Gammarus* and *Hyalella* (Schindler et al. 1992). The annual winter pH depression in LRL (Kratz et al. 1987) as well as the low calcium concentration may be responsible for the lack of acid-intolerant taxa in the lake.

A wide range of responses to acidification was detected for the most common benthic taxa collected on artificial substrates

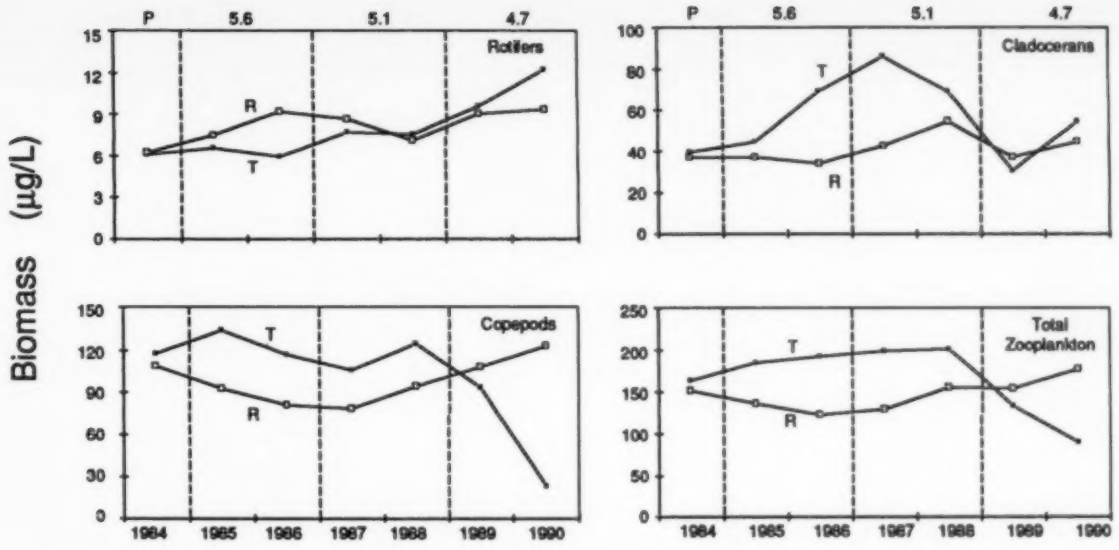

FIG. 9. Mean annual biomass of the zooplankton community and its three major components during ice-free periods in the LRL treatment and reference basins.

TABLE 4. Responses to acidification by the most common littoral macrobenthos (excluding Chironomidae) colonizing artificial substrates in the fall of each year, LRL. Increases or decreases were assigned when differences between the treatment and reference basins exceeded the standard error of measurement for both years at a target pH. 0 = no response; − = negative response; + = positive response; — = not collected in the treatment basin.

	pH 5.6	pH 5.1	pH 4.7
Insect larvae			
Ephemeroptera			
Leptophlebia	0	—	—
Caenis	0	−	−
Trichoptera			
Leptoceridae	0	0	0
Oxyethira	0	+	+
Polycentropus	0	0	0
Odonata			
Enallagma	0	0	+
Libellulidae/Corduliidae	0	0	0
Coleoptera			
Dineutus	0	0	+
Diptera			
Ceratopogonidae	0	0	+
Other macroinvertebrates			
Amphipods			
Crangonyx gracilis	0	0	0
Spaeriid clams	0	0	0
Water mites	0	0	0
Total macroinvertebrate abundance	0	0	0
Number of macroinvertebrate taxa	0	0	0

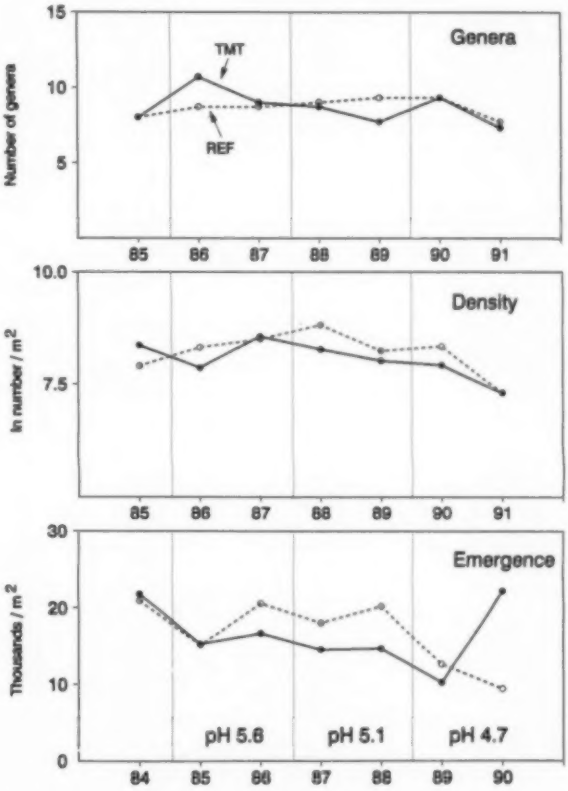

FIG. 10. Number of genera and abundance of Chironomidae larvae in the spring and total annual Chironomidae emergence at the 4-m sites in the LRL treatment and reference basins. In the top two panels, spring measurements reflect effects of the pH of the previous autumn.

TABLE 5. Responses to acidification by common Chironomidae genera and *Chaoborus* in sublittoral (4 m) habitats, LRL. Responses for densities of common genera of Chironomidae larvae and total number of general were determined from dredge samples collected in spring and annual emergence totals of Chironomidae and *Chaoborus*. Increases or decreases were assigned when differences between the treatment and reference basins exceeded the standard error of measurement for both years at a target pH. 0 = no response; − = negative response; + = positive response.

	pH 5.6	pH 5.1	pH 4.7
Spring larval density			
Chironomini			
Chironomus	0	0	0
Dicrotendipes	0	0	0
Endochironomus	0	0	+
Microtendipes	0	0	−
Pagastiella	0	0	0
Polypedilum	0	0	0
Pseudochironomus	0	−	−
Tanytarsini			
Parantanytarus	0	−	−
Tanytarus	0	0	0
Tanypodinae			
Procladius	0	0	0
Orthocladiinae			
Psectrocladius	0	0	+
Chaetocladius	0	+	+
Total Chironomidae	0	0	0
Number of Chironomidae genera	0	0	0
Annual emergence			
Chironomidae	0	0	(+)[a]
Chaoborus	0	0	+

[a]Increase occurred only in second year of treatment step.

(Table 4). Many, including *C. gracilis*, showed no differences in abundance between the two basins throughout the experiment. The only negative responses observed were for the two common mayfly genera in LRL, *Caenis*, which declined at pH 5.1 and virtually disappeared at pH 4.7, and the less common *Leptophlebia*, which was not found after acidification to pH 5.1. Four taxa increased in abundance at lower pH. *Oxyethira*, a microcaddisfly, showed a positive association with the extent of benthic algal mat development (Webster et al. 1992). Increases also were detected in predaceous larvae of the damselfly *Enallagma* and beetle *Dineutus*, as well as dipterans from the family Ceratopogonidae.

Spring sublittoral (4.0 m) Chironomidae assemblages in the two basins did not differ either in number of genera or total density throughout the experiment (Fig. 10). Interbasin differences in the abundance of the most common genera (12 from total of 24) collected in dredge samples (Table 5) occurred primarily at pH 4.7; these included declines in *Microtendipes*, *Pseudochironomus*, and *Paratanytarsus* and increases in *Psectrocladius* in the treatment basin relative to the reference basin. Occasional differences were observed at intermediate acidification steps, but most of these did not persist through the end of the experiment.

Annual totals for Chironomidae collected in emergence traps likewise showed no interbasin differences except for a large

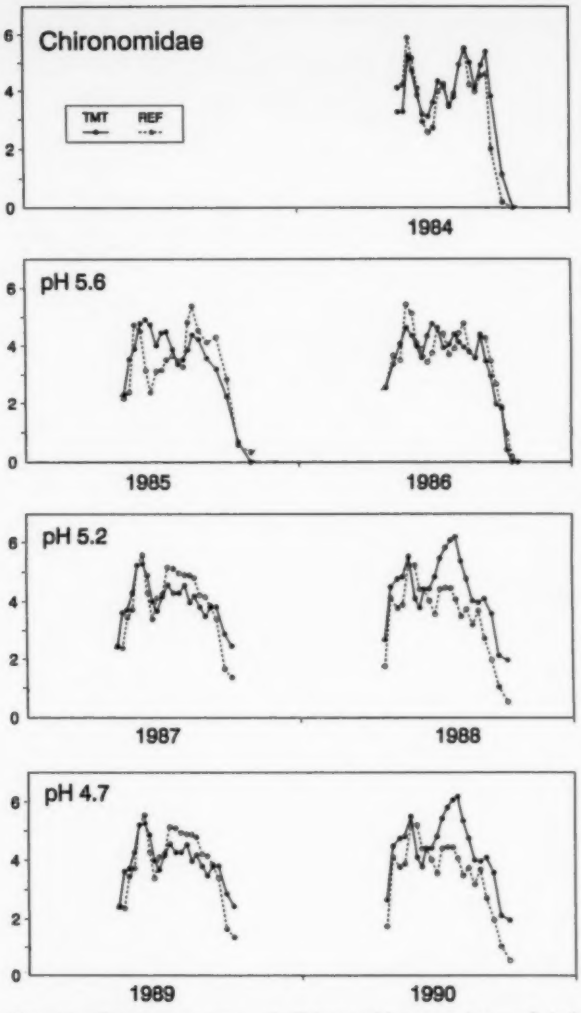

FIG. 11. Emergence rates of Chironomidae (number·m^{-2}·d^{-1}) throughout the ice-free season at the 4-m sites in the LRL treatment and reference basins. Data represent log-transformed [ln(n + 1)] mean values from three sites per basin in most years (one in 1984 and two in 1985).

increase in the treatment basin relative to the reference basin in year 2 at pH 4.7 (Fig. 10). Weekly emergence patterns for Chironomidae (Fig. 11) do suggest marked interbasin differences following acidification, but a finer scale of taxonomic identification is required to resolve them. Annual emergence of *Chaoborus* spp. was higher in the treatment basin than in the reference basin at pH 4.7, corroborating results from pelagic sampling summarized in Table 3. In contrast with those results, however, the increase in collections by emergence traps was not detected until the last acidification step.

Most of our preacidification predictions regarding changes in the benthic macroinvertebrate community (Brezonik et al. 1986) were not observed, and some observed responses had not been predicted. Mayflies were absent from the treatment basin at pH 4.7 as predicted, but declines at pH 5.1 occurred one step lower than that predicted (pH 5.5). Based on samples analyzed to date, we have no evidence for predicted declines in oligo-

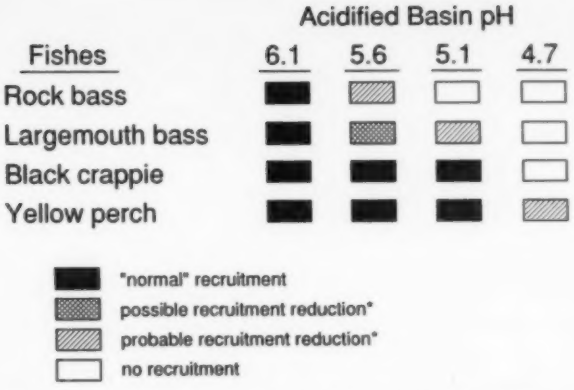

FIG. 12. Population responses of rock bass, largemouth bass, black crappie, and yellow perch in LRL at successive pH treatment values. Possible = a minor change from reference conditions; probable = a major or statistically significant change from reference conditions.

chaetes, leeches, sphaeriid clams, *Polycentropus*, water mites, or number of macroinvertebrate genera (Tables 4 and 5). Sensitivity of the Tanytarsini to acidity was supported by reduced density of *Paratanytarsus* in spring after acidification to pH 5.1. A more complete analysis of the chironomid assemblage is required to fully evaluate the response of these organisms to the LRL acidification.

Our results provide evidence that indirect effects are important in structuring the responses of many macroinvertebrates to acidification. The marked increase of *Oxyethira* was an unexpected indirect response to algal mat development. The increase in acid-tolerant predaceous insect larvae is a well-documented response to the loss of fish populations following lake acidification (Eriksson et al. 1980; Bendell and McNicoll 1987; Stenson and Eriksson 1989). We did not predict such an increase in LRL mainly because the dominant benthivore in LRL, yellow perch, was not expected to be affected by the acid treatment. However, we did observe an increase in damselfly and beetle larvae at pH 4.7, concomitant with the increase in *Chaoborus* and Chironomidae emergence, the latter only in year 2 at pH 4.7. These results emphasize the importance of experimental manipulations in detecting indirect effects of acidification (Webster et al. 1992).

Fish

Field observations suggested that rock bass was the most sensitive of the major fish species in LRL (Eaton et al. 1992). Recruitment of rock bass was probably reduced at pH 5.6, and complete recruitment failure occurred in the treatment basin population at pH 5.1 (Fig. 12). Recruitment was reduced in largemouth bass at pH 5.6 or 5.1, and complete recruitment failure in largemouth bass and black crappie occurred at pH 4.7. The first sign of reduced recruitment in yellow perch also occurred at pH 4.7 (Fig. 12). These responses suggest that recruitment is generally reduced at pH values above those that result in complete recruitment failure and population losses. The pH values at which reductions in recruitment were observed in LRL generally were higher than the critical values inferred from fish survey studies, which employed presence–absence criteria to describe effect pH values (Magnuson et al. 1984b). The results from experimental approaches such as the LRL study thus give different indications of how sensitive fish are to pH than do lake surveys, which generally do not consider recruitment reduction.

Our results also indicate a different order of sensitivity to pH among the LRL fish species than that given by the lake survey results summarized by Magnuson et al. (1984b). The lake surveys indicate that black crappie populations are more sensitive to acidification than rock bass, the species identified to be most sensitive by LRL field work. Other studies also suggest that rock bass are more sensitive to acidification than indicated by published survey results (Snucins and Shuter 1991). The differences may be attributable to variation in pH sensitivity between populations or to the operation of different ecosystem-linked mechanisms (e.g., the presence of additional toxicity factors). Our inability to observe a reduction in recruitment of black crappie above the pH at which complete recruitment failure occurred may be related to the small size of the population. Estimated black crappie populations (age 1 and older) in the two basins consistently comprised fewer than 90 individuals.

Autumn age 0 largemouth bass abundances and estimated recruitment rates (Swenson et al. 1989) for the two basins during the pH 5.1 treatment suggested that overwinter survival rates were substantially lower in the treatment basin (0.0019) than in the reference basin (0.0083). To improve understanding of the long-term effect of the pH 5.1 treatment on largemouth bass production, a Leslie matrix analysis (Leslie 1945) was used to predict future population abundances in the two basins if the pH 5.1 treatment and the related reduction in first-year survival had been continued. Simulations were run with only first-year survival varying between basins. Reference basin age structure, average survival of recruited age classes (S = 79%), and the average number of young produced per spawning female (49 fall fingerlings) in the two basins were used in the simulations. The results indicate that the estimated first-year survival rates in the reference basin would support population growth, but first-year survival rates in the treatment basin resulted in a population loss to fewer than 10 individuals in 20 yr.

Reduced production rates and complete reproduction failure appear to be the result of several mechanisms that directly or indirectly control recruitment. Observation of largemouth bass nests (by snorkeling) showed that nesting activity was not reduced at lower pH, but direct pH effects on embryo–larval stages of development appear to be important. The percentage of nests in the treatment basin that produced swim-up fry fell below the reference basin at pH 5.1. At pH 4.7, no nests were observed to produce swim-up fry. Although the percentage of nesting success was lower in the treatment basin at pH 5.1, adult abundances, the total number of nests, and the number of young were higher, making interpretation of the importance of reduced nesting success to recruitment difficult. No differences in survivorship were observed in in situ experiments conducted through 7 d post-hatch with largemouth bass embryos obtained from treatment and reference basin nests during the pH 5.1 phase.

The secretive habits of rock bass fry and young prevented us from defining their abundances accurately. Therefore, they could not be used to determine whether direct embryo–fry mortality was the cause of the reduced recruitment observed for rock bass. Abundances of black crappie fry did not appear to be influenced above pH 4.7, the treatment during which black crappie fry were completely absent from the field samples. Although yellow perch larval survival was reduced at pH 4.7, growth was also significantly lower and the changes coincided

TABLE 6. Summary of LRL responses at pH 5.6.

Component	Definite change	Possible change	No change
Phytoplankton		Increased primary production (year 2)	Average and peak Chl *a*
Pelagic zooplankton	Of 34 dominant taxa, 7 declined and 2 increased		Total biomass
Littoral zooplankton	Of 9 dominant taxa, 2 increased and 1 decreased; increased abundance/biomass		6 taxa
Benthos	Missing or asynchronous peaks in Chironomidae emergence		Total density and number of genera
Algal mat	Large increase in areal coverage		
Fish	Reduction in survival of rock bass and largemouth bass year classes	Average size of young-of-the-year largemouth bass	Condition of adults of all species
Major ions	Small increase in Ca; decrease in ANC; increase in sulfate		Na, K, Mg, Cl
Minor and trace metals	Increase in dissolved Mn, Fe; increase in yellow perch body burden of Hg	Increase in Fe	Al, Cd, Cu, Pb, Zn
Nutrients			Means and seasonal distribution of inorganic and total N and P, TOC, and SiO_2
Processes	Increase in sulfate reduction and ANC generation	Microbial density of leaf litter	Litter decomposition rates
Physical conditions	Small decrease in color; increase in transparence; decreased spring heat absorption		Turbidity

with reductions in total zooplankton biomass and copepod biomass, the dominant prey of larval yellow perch. Therefore, it is not clear whether the response was the result of a direct effect of acid treatment or was linked to the observed changes in the forage base. Larval yellow perch are very sensitive to handling, and no in situ experiments were conducted at pH 4.7 with this species. Laboratory bioassays conducted with simulated LRL water chemistry conditions suggest a direct response to pH 4.7 (McCormick et al. 1989). Nonetheless, reduced growth and survival of some yellow perch through the whole summer suggests that the response was at least partially the result of changes in the forage base.

The mechanisms responsible for high overwinter mortality in age 0 largemouth bass were delineated through a 131-d laboratory study designed to measure the combined influence of winter temperature (4°C), pH, and Al concentrations similar to those measured in LRL on young-of-the-year largemouth bass. This study demonstrated that first-year largemouth bass survival was significantly lower at pH 5.0 with 30 μg (inorganic monomeric) Al/L than under simulated reference basin conditions (McCormick and Jensen 1992). Although we do not have corresponding laboratory and field information for rock bass, we suggest that the combination of low temperature, pH, and Al stress also may have caused the reduction in rock bass recruitment at pH 5.6.

Observations of feeding activity, diet analysis, measurements of fish growth rates, and estimates of fish condition factors all fail to support hypotheses that pH-associated changes in lower trophic groups significantly influenced the fish species complex, except at pH 4.7 when reductions in growth and survival of larval yellow perch coincided with reductions in their major prey. Direct observation of the foraging behavior of first-year largemouth bass and analysis of their diets (Swenson et al. 1989) suggested that a slight shift to feeding on smaller prey occurred at pH 5.1. This behavioral change was associated with the development of a dense algal mat over the lake bottom and throughout the water column between littoral rooted macrophytes where young bass congregated in the absence of the thick algal growth. However, estimates of the number of prey attacks per minute and condition factor suggest that the shifts in distribution or diet did not cause significant growth or survival differences. Although growth was slower in the treatment basin in 1987 (year 1 at pH 5.1), the difference appeared attributable to higher densities of young bass. Growth of young largemouth bass was similar in the two basins during 1988 when they had approximately equal densities. Condition factors calculated for all species and age groups (except larvae) did not differ between basins during any of the pH treatments.

Fish responses observed during the acidification of LRL are generally similar to those for Lake 223 (ELA). Recruitment failure was identified as the cause of population declines in that cold-water habitat. Although condition factors of lake trout (*Salvelinus namaycush*) declined prior to observed reductions in recruitment rates (as the abundance of important forage species declined), lake trout and white sucker (*Catostomus commersoni*) abundances and growth rates increased or remained unchanged during most of this period (Mills et al. 1987). Increased competition for food was suggested as the cause of

TABLE 7. Summary of LRL responses at pH 5.1.

Component	Definite change	Possible change	No change
Phytoplankton		Seasonal variance in Chl *a*; increased primary production	
Pelagic zooplankton	14 taxa decreased and 4 increased; total cladocean biomass increased	Lower species diversity	Net grazing; total copepod or zooplankton biomass
Littoral zooplankton	5 taxa increased in year 1 (mat effect); 1 almost gone; 1 new taxon; biomass and abundance increased both years		
Benthos	1 taxon increased (indirect effect of algal mat); loss of *Leptophlebia* (mayfly)	*Caenis* (mayfly) abundance declined	Total density and number of genera
Algal mat	Further increase in areal coverage		
Fish	Reduced year-class strength of black crappie, rock bass, and largemouth bass	Rock bass year class lost; smaller average size of young-of-the-year largemouth bass	Condition of adults of all species
Major ions	Further increase in Ca and sulfate and decrease in ANC	Increase in Mg, K	Na, Cl
Minor and trace metals	Further increase in dissolved Mn, Fe; increase in Al (avg. 28 μg/L)	Increase in Cd, Zn during 2nd year	Cu, Pb
Nutrients		Lower TOC and decrease in TN (year 2)	Means and seasonal distribution of inorganic and organic N, P, and SiO_2
Processes	Further increase in sulfate reduction; decrease in oak litter decomposition and bacterial colonization/activity on leaves	Decrease in birch leaf decomposition	
Physical conditions	Decrease in color; increase in transparency	Increase in variance of turbidity	

the decline in condition. Mills et al. (1987) indicated that the increased abundance of white sucker was unexpected and may have been related to development of an algal mat that appeared to provide food and cover for first-year fish. We observed young yellow perch feeding and taking cover in the algal mat that developed in LRL at pH 5.1 when indices of yellow perch survival and recruitment were higher in the treatment basin (Webster et al. 1992). As the Lake 223 experiment progressed, lake trout and white sucker recruitment rates declined before any observed reductions in growth rates (Mills et al. 1987). This suggests that recruitment failure was not linked to the shifts in population abundances observed within lower trophic levels of Lake 223.

The lack of a clear link between fish population responses and changes occurring at lower trophic levels in both the Lake 223 and LRL experiments can be explained in several ways. Although both investigations might be considered long term, the time frames may not have been adequate to describe the influence of this type of indirect mechanism. Experiments conducted at one test pH for extended periods could provide different results. However, comparison of our results with those developed through lake surveys shows that experimental manipulations identified important reductions in fish production rates occurring at pH values above those that result in total extirpation of populations from ecosystems. Finally, the LRL experiment did not suggest the occurrence of substantial reductions in the overall productivity of lower trophic levels at pH treatments higher than those affecting fish. Considering that freshwater fish possess relatively limited capacity for adaptation through natural selection and include life stages that are sensitive to environmental changes, it should not be surprising that fish are as sensitive to direct mechanisms as the organisms they prey upon. Furthermore, the diet of freshwater fish is characterized by plasticity (Larkin 1956), increasing the potential for behavioral adaptation to shifts occurring in lower trophic levels. Shifts in diet were observed in both Lake 223 and LRL and suggest the value of this adaptive mechanism in responding to system change.

Summary and Conclusions

Chemical and biological effects were observed at each of the three pH levels of the experiment (Tables 6–8), and the changes became more pronounced and more numerous as the pH was lowered. The availability of data for subsequent stages of acidification often permitted us to conclude that a small change observed in an earlier stage was the beginning of a longer trend and indeed a real effect. With regard to chemical responses, all

TABLE 8. Summary of LRL responses at pH 4.7.

Component	Definite change	Possible change	No change
Phytoplankton	Loss of blue-green species; increased photosynthetic sulfur bacteria in hypolimnion	Increase in primary production	Chl *a*
Pelagic zooplankton	20 taxa declined; 8 increased; species diversity decreased; total biomass declined; community significantly changed		
Littoral zooplankton	1 taxon gone; 3 declined; 1 increased; overall abundance and biomass increased	1 taxon increased	5 taxa unchanged
Benthos	Loss of *Caenis*; increase in predaceous insect larva and *Chaoborus* emergence	Increase in Chironomidae emergence	Total density and number of genera
Alga mat			Areal coverage
Fish	Year-class failures of rock bass and largemouth bass; young-of-the-year yellow perch did not survive in 2nd summer at pH 4.7		
Major ions	Ca up to ~180 μeq/L; Mg, K up slightly; sulfate up to 147 μeq/L; pH depressed in top 10 cm of sediment porewater	Base cation depletion in surface sediments	Na, Cl
Minor and trace metals	Mn increases continue; Al up to 38 μg/L; small increases in Cd, Pb, Zn	Decrease in dissolved Fe	Cu
Nutrients	TOC, TN down significantly	Small increase in summer avg. SRP, NH_4^+, NO_3^-, SiO_2	Means and seasonal variation of TP
Processes	Small decline in birch; large decline in oak decomposition; decline in microbial colonization	Cycling of allochthonous leaf carbon	
Physical conditions	Color		

major ions except Na^+ and Cl^- changed, dissolved levels of the three minor cations (Al, Fe, and Mn) increased, TOC and color decreased, and dissolved concentrations of some trace metals increased slightly. In addition, bioaccumulation of trace metals was affected, and some major biogeochemical processes, including sulfate reduction, N_2 fixation, and organic matter decomposition, were significantly affected.

Some biotic responses appeared to be the result of direct physiological effects of pH (or possibly related chemical changes) on organisms, but others (e.g., those that did not occur until year 2 at a target pH) probably were indirect effects of changes in food resources or habitat. The appearance of a mat of filamentous green algae in the littoral zone of the treatment basin was the most visible biological effect of acidification. While it varied in extent and density each year, it was a common feature of the treatment basin but was absent (or nearly so) from the reference basin during the experimental period. Over the pH range 6.1–4.7, a major shift occurred in pelagic zooplankton species composition, as well as in the phytoplankton community. Smaller shifts occurred in the littoral zooplankton and benthic macroinvertebrate communities. In general, acidification simplified the LRL food web, as acid-sensitive taxa disappeared and fewer acid-tolerant species replaced them. All four major species of fish were affected by acidification, but the pH of first impact ranged from 5.6 for rock bass to 4.7 for yellow perch. The mechanism of impact also varied among species, ranging from decreased nesting success and increased first-year overwintering mortality for largemouth bass to poor survival of young-of-the-year for yellow perch. Acidification did not significantly affect adult fish, except for the small population of rock bass.

In contrast with laboratory (microcosm) and field enclosure (mesocosm) experiments, whole-basin manipulations like the acidification of LRL's north basin provide appropriate spatial and temporal scales to assess the direct and indirect effects of culturally derived stresses on aquatic ecosystems. On the other hand, such studies are costly and time-consuming, and often pose difficult logistical problems. In particular, the difficulty in replicating such manipulations complicates the interpretation of data and often means that special techniques and supplemental information must be used to draw proper inferences from observational data. Nonetheless, such manipulations are invaluable in identifying critical mechanisms of aquatic ecosystem responses to stresses and sensitive life stages or processes. For example, embryo and larval life stages of fish are widely accepted as the most sensitive to direct effects of acidification. However, we found that the interplay between pH stress and overwinter conditions controlled year-class formation for large-

mouth bass and probably for rock bass in LRL. Neither routine laboratory bioassays nor synoptic surveys across a pH gradient could have identified this as a mechanism explaining the disappearance of these fish from acidic lakes. Similarly, the whole-basin experiment demonstrated that leaf litter decomposition is inhibited at lower pH, and through a combination of laboratory and small-scale field studies, we were able to show that the severity of impacts is related to the quality (C:N ratio) of the litter being decomposed and to changes in leaf litter microbial community structure and activity.

The pH manipulation at LRL verified a wide range of chemical, biotic, and bioprocess responses to acidification that had been hypothesized from lake surveys and laboratory studies. In some cases (e.g., elevated Cd in fish of acidic lakes), the expected trend was not verified, and further studies are needed to evaluate causes of the different response. In other cases, the response at LRL was different from that reported for other lake pH manipulations (e.g., differences in pH effects on nitrification, decomposition, and numerous zooplankton species in LRL and ELA lakes). These results point to the complexity of nature and the importance of replicating manipulations in different geographic areas.

Predictions of changes in major ions, minor and trace metals, and IAG in the treatment basin made from enclosure and laboratory experiments were semiquantitatively correct. As predicted, IAG increased with H^+ loading, at least during the first half of the experiment. Sulfate reduction accounted for the loss of about half of the added sulfuric acid. Ion exchange of sediment cations and water column H^+ was important in IAG but resulted in acidification of the surficial sediments. This may have diminished the lake's ability to counteract further H^+ inputs, and the acidic sediments will exert an alkalinity demand during the recovery stage.

Biotic responses proved more difficult to predict. Limited information on which to base predictions was available for some organisms; for others the information was conflicting. In general, we were unsuccessful in predicting indirect responses caused by changes in food resources or habitat, such as the spread of the algal mat in year 1 at pH 5.1, which allowed increased abundance of two benthic invertebrate species and may have afforded greater protection to young-of-the-year yellow perch. Many changes in zooplankton and benthic invertebrates involved an interplay between food-web conditions and pH rather than direct pH effects. Population parameters generally were more sensitive to pH than were community parameters for zooplankton; effects on total biomass were delayed. While species composition changed gradually from pH 6.1 to 4.7, a major shift in zooplankton community parameters was obvious only at pH 4.7.

Fish production in LRL was reduced at pH values above those defined as critical by traditional laboratory bioassays or by field studies based on presence-absence criteria. Moreover, year-class formation in three of the common LRL fish species was reduced at pH values above those that resulted in complete year-class failures. The capacity to support sportfishing thus is diminished in lakes undergoing acidification before fish populations are lost altogether. Comparison of our results with published critical values (e.g., Magnuson et al. 1984a) suggests that production is affected ~0.5 pH unit above the values that cause complete year-class failure.

Acidification generally did not affect adult fish in LRL, except for the population of rock bass, which most likely was killed directly by water quality conditions (interaction of pH, Al, low Ca^{2+}, and possibly low winter temperatures). For the other three major fish species, juvenile stages were most sensitive to acidification. Finally, evidence developed through the LRL study and overwintering studies on smallmouth bass (*Micropterus dolomieu*, formerly *M. dolomieui*) (Shuter and Ihssen 1991) and largemouth bass (McCormick and Jensen 1992) suggests that populations of warmwater species at the northern edge of their range may be especially sensitive to acidification.

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LETTERS AND COMMENTS/LETTRES ET COMMENTAIRES

Comment on "Use of Risk Analysis to Assess Fishery Management Strategies: A Case Study Using Orange Roughy (*Hoplostethus atlanticus*) on the Chatham Rise, New Zealand" by R.I.C.C. Francis

Francis (1992) presents a method for calculating risk to the fishery of alternative management plans. The key element in the method presented is a Monte-Carlo procedure for calculating the probability density function for the virgin biomass (denoted B_0). This probability density function is then used to calculate risk to the fishery. While the objective of this paper is admirable, we find a number of problems in the computational procedure he describes. There is a well-established methodology for risk analysis — it is called Bayesian decision theory and there are many books published in the area (e.g., Raiffa 1968; Berger 1985). Bayesian analysis has been frequently applied in fisheries (e.g., Ludwig and Walters 1982; Mangel and Clark 1983; Deriso and Parma 1988; Fried and Hilborn 1988; Nakamura et al. 1989; Parma and Deriso 1990a, 1990b; Zeh et al. 1990; Greiger and Koenings 1991; Hilborn and Walters 1992). If the procedure Francis presents is superior to Bayesian methods, he should have compared his results with those obtained from a Bayesian analysis. When a new procedure is proposed in an area where an accepted method is widely used, the author should compare his new procedure with the existing one. While Bayesian decision analysis had not been used for exactly the same problem Francis considered, the theory is well developed and used in many related problems.

Part of the computation procedure involves numerically constructing a cumulative distribution, fitting a Johnson's distribution to this cumulative distribution, and then taking the first derivative to obtain what Francis considers a probability density function for the parameter of interest, virgin biomass. We have implemented Francis's procedure and find that for data sets for which Francis's method has been applied (Cordue and Francis 1992), the cumulative distribution does not reach an asymptote of 1.0. It is not at all clear how to interpret Francis's method when the cumulative distribution does not reach 1.0.

Francis's method uses only the point estimate of the parameter B_0 and makes no use of the variability in the data. Francis (1992), for example, estimated the variance from the sampling error in surveys and suggested fitting the variability in the data, but in practice (Cordue and Francis 1992; Cordue et al. 1992), this has not been done. Figure 1 shows two data sets and best-fit models. Data set A corresponds exactly with the deterministic fit to the model, while data set B shows very high variance. However, since the best fit B_0 for each model is the same, Francis's method would assign alternative hypotheses about B_0 exactly the same probability under the two data sets. While the variance should clearly be higher for data set B, the variance as the procedure has been implemented has been derived from an understanding of the survey process used to derive the index of abundance and makes no use of the year-to-year variability in the data.

Finally, and most importantly, Francis argues that the product of his method can be used in decision analysis. The crux of this argument is that the distribution that emerges from his method reflects the relative credibility of alternative values of virgin biomass. For two competing hypotheses about virgin biomass, the relative probabilities of the hypotheses that are used for the risk analysis should reflect the relative support that the data provide for the alternative hypotheses about virgin biomass.

In Bayesian analysis, the relative credibility of the hypotheses depends upon two components: the probability of observing the data if the hypotheses are true and the prior probability of the hypotheses. Most critics of Bayesian estimation attack the sensitivity of the final results to prior probabilities. We show below that the basic likelihood (the probability of observing the data

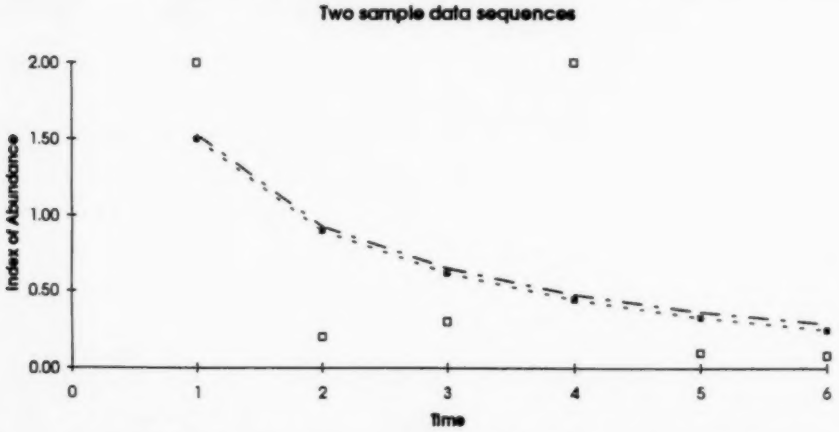

FIG. 1. Two data sets that each have an estimated K of 1.5. Data set A (solid squares) is completely consistent with a deterministic model. Data set B (open squares) is highly variable. The two lines show the best fits to the two data sets.

if the hypothesis is true) calculation differs greatly from Francis's result and that the differences between Francis's method and Bayesian analysis are not due to the use of prior probability distributions.

Edwards (1972) argued that the relative credibility of alternative hypotheses is simply the ratio of their likelihoods. For instance, for some set of data D, and two hypotheses A and B, the relative credibility of hypothesis A to hypothesis B would be

$$\frac{L(\mathrm{D}|\mathrm{A})}{L(\mathrm{D}|\mathrm{B})}$$

where $L(\mathrm{D}|\mathrm{A})$ is the likelihood of D given hypothesis A.

The likelihood is rather simply calculated. Indeed, Francis calculates the relative likelihood of different virgin biomasses as part of his procedure. His method appears to be an elaborate computational procedure which is much more efficiently, and as we will see below, more reliably, estimated by a much simpler calculation. In the paragraphs below, we use a numerical example to show that Francis's method produces answers that are contrary to relative likelihood.

Francis used an age-structured model fit to an index of abundance, and we have no criticism of the model — indeed, we have been using such models for a number of years (e.g., Hilborn 1990). However, for illustration, we will use a much simpler model that permits much quicker computation for anyone wishing to replicate our results. Similar results are obtained when using full age-structured models.

Using the simple deterministic discrete time Schaefer model (Hilborn and Walters 1992), the population dynamics are

$$(1)\qquad B_{t+1} = B_t + rB_t\left(1 - \frac{B_t}{K}\right) - C_t$$
$$C_t = B_t\mu$$

where B_t is the stock biomass at the beginning of year t, I_t is an index of abundance for year t, C_t is the catch in year t, r is the intrinsic rate of growth, K is the virgin biomass, and u is the harvest rate. We assume that an index of abundance I_t is observed each year and that this index is proportional to the stock biomass with the parameter q being the index of proportionality. Further, we assume that the observation process is lognormally distributed:

$$(2)\qquad I_t = qB_t\exp(w_t)$$
$$w_t = N(0,\sigma_w).$$

We have performed a number of numerical experiments with this model and find that when the data set is highly informative, Francis's method produces results nearly identical to likelihood; however, when the data are uninformative, the results are very different from likelihood. We therefore will provide a numerical demonstration of these differences for a highly uninformative data set that illustrates the failings of Francis's method.

We assume that r is known and (equal to 0.2) and that the standard deviation of the observation error (σ_w) is also known and equal to 0.2. Thus, the two unknown parameters are K, the virgin biomass and the parameter of interest, and q which is a nuisance parameter. This is analogous to Francis's age-structured model where the two unknown parameters are B_0 and q. We assume that we have 5 yr of catches and index of abundance data and that $u = 0.1$, which provides for a rather low rate of exploitation and therefore an uninformative data set, and that the real K (noted as K^{real}) is 1.5. Using this model and these parameters, we can generate any number of simulated data sets for any value of K^{real}. The short data series and the low harvest rate make this an uninformative data set.

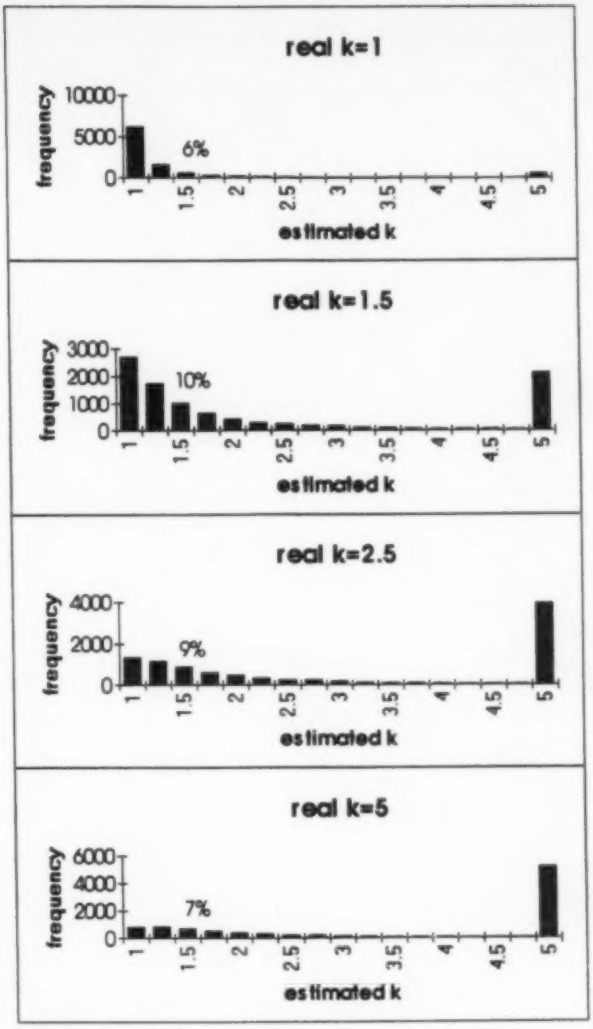

FIG. 2. Frequency distribution of estimated K values for four possible true K values.

The maximum likelihood estimate of K is obtained in the following way. Given the vector of catches ($\boldsymbol{C}$) and abundance indices of ($\boldsymbol{I}$) and any value of K, the equations above can be used to generate a vector of predicted biomasses ($\hat{\boldsymbol{B}}$). Assuming the log normal error structure as shown above, the maximum likelihood estimate of q is

$$(3)\qquad \hat{q} = \exp\left[\frac{1}{n}\sum_t \log\left(\frac{I_t}{\hat{B}_t}\right)\right].$$

The predicted index of abundance is thus

$$(4)\qquad \hat{I}_t = \hat{q}\hat{B}_t$$

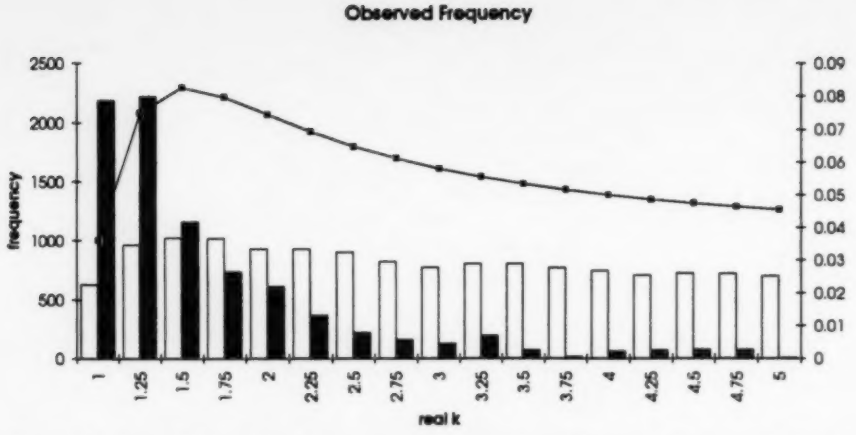

FIG. 3. Relative credibility of hypotheses generated by Francis's confidence distribution method (solid bars), the frequency of K estimates near 1.5 from Monte-Carlo trials (open bars), and the relative likelihood of a deterministic sequence of index of abundance (line).

and the negative log likelihood (ignoring constants) is proportional to

$$(5)\quad L(\hat{\mathbf{B}}) = \sum_t (\log(I_t) - \log(\hat{I}_t))^2.$$

We now show results of a numerical experiment using the above assumptions to compare Francis's method with traditional likelihood. Assume that we have a data set for which the estimate of K is 1.5. As Francis's method does not use anything other than this estimate, the amount of variation in the actual data is unimportant. Of course, σ_w is used in generating the Monte-Carlo data sets. We now compute three quantities. First, we use Francis's method and simulate 10 000 Monte-Carlo replicates at each level of K^{real} between 1 and 5 in steps of 0.25 using equations 1 and 2. For each Monte-Carlo replicate, we determine the maximum likelihood estimate of K by direct search over intervals of $0.25K$. Thus, for any specific simulated level of K^{real}, we will have the number of the 10 000 Monte-Carlo replicates where $\hat{K}$ falls within the intervals 0.875–1.125, 1.125–1.375, 1.375–1.625, etc. Figure 2 shows typical results for K^{real} of 1, 1.5, 2.5, and 5. Printed in the frequency distribution are the percentage of trials in which $\hat{K}$ would have been between 1.375 and 1.625 which is a Monte-Carlo estimate of the likelihood of the "data" ($\hat{K} = 1.5$) given K^{real}. If K^{real} had been 1, then in 6% of the trials, $\hat{K}$ would have been about 1.5, while if K^{real} was 1.5, then in 10% of the trials, $\hat{K}$ would have been about 1.5. Even when K^{real} was 2.5 or 5, for 9 and 7% of the trials, $\hat{K}$ would have been about 1.5. We can see from Fig. 2 that regardless of what the K^{real} is, $\hat{K}$ would have been 1.5 between 5 and 10% of the time. If K^{real} is 5.0 the probability of observing the data ($\hat{K} = 1.5$) is 70% as likely as if K^{real} is 1.5. Thus the Monte-Carlo experiment indicates that given a data set with the $\hat{K} = 1.5$, we must conclude that the data support the hypothesis $K = 5$ almost as well as they do the hypothesis $K = 1.5$.

There should be no surprise here. The data were uninformative about K, simply because there was little exploitation and the time series was short (see Hilborn 1979 or Hilborn and Walters 1992 for fuller explanation). To compare these Monte-Carlo likelihoods with direct analytic likelihoods, we simulated a deterministic trajectory (no observation error) with $K^{\text{real}} = 1.5$ (trajectory in Fig. 1) and then computed the likelihood of different K values between $K = 1$ and $K = 5$ using equations 3–5. By using deterministic data, we have the maximum difference in credibility between alternative K values, given that the point estimate is $K = 1.5$. If we had used a more varible data set whose K estimate was 1.5, the likelihood distribution would have been even flatter.

Figure 3 shows the likelihood of the deterministic data series (solid line), the probability of $1.375 \leq \hat{K} \leq 1.625$ (open bars), and the estimated density from Francis's method (solid bars). The cumulative distribution, the heart of Francis's method, flattens out quickly at about $K = 2.5$, so that the first derivative (solid bars) is small and close to zero. Francis's method says, therefore, that there is little chance that the real K was greater than 2.5. On the other hand, we see that both the likelihood and the actual frequency of estimated K's show that even if K is 5, we are at least half as likely to obtain an estimated K of 1.5 as we are if the true value of K is 1.5. Also, the cumulative proportion of estimates greater than or equal to 1.5 does not reach an asymptote of 10 000, but rather 8340.

We have performed many Monte-Carlo experiments such as described above. As a general result, if the data set is highly informative, which would mean high harvest rates or low variance, there will be little difference between Francis's method and likelihood. However, when the harvest rates are low or variance high, and data uninformative, Francis's method says that the probability that K is large is much lower than likelihood theory. Since the likelihoods are vastly easier to calculate, we see no circumstance where Francis's method should be employed, at least for estimation of K or analogous parameters.

First we assert that if Francis's method provides different answers that likelihood, then likelihood is correct. Thus the failure to correspond to relative likelihoods we view as a failure of his method. While Francis' states "The derivative of this curve is a probability distribution for B_0" (Francis 1992, p. 924), we believe that this example demonstrates that it is the wrong probability distribution. We see two reasons for this. First, the method Francis uses is as he says, analogous to a method in Mood and Graybill (1963), but the method described in Mood and Graybill is for determining confidence intervals, not probability of the parameter of interest. Second, when the

data are uninformative enough that there is a long, or infinite right-hand tail on the likelihood, the cumulative probability function will flatten out and its first derivative will be zero, Thus, any B_0 value in this range of flat likelihood will be assigned no probability, even if there is a substantial chance of obtaining a point estimate close to that seen in the data.

In summary, we believe that the confidence distribution method proposed by Francis should not be employed for estimating virgin stock size. Regardless of whether one takes a strict likelihood or a Bayesian perspective, Francis's method provides quite different answers when data sets are uninformative. When data sets are informative, his procedure provides similar answers to likelihood or Bayesian methods at great computation expense — Ray Hilborn, Ellen K. Pikitch, Murdoch K. McAllister, and Andre E. Punt, *School of Fisheries, University of Washington, Seattle, WA 98195, USA.* (JB723)

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Estimation of Risk: A Response to Hilborn et al.

I am sorry to find little useful in the comments of Hilborn et al. (1993). In much of what they say, they either misunderstand or misrepresent the aims and methods of the risk analysis procedure of Francis (1992).

The bulk of their paper is devoted to an analysis that is concerned with different hypotheses about the virgin biomass, B_0 (which they call K, or sometimes, k). It is hard to see the relevance of these calculations. The aim of the procedure described by Francis (1992) is to estimate the biological risk associated with various alternative management actions. As an intermediate result, a probability distribution for B_0 is calculated. However, the primary aim is to estimate risk. How good this and alternative procedures are will be found by investigating how well they perform as estimators of risk. Precisely this evaluation is made by Cordue and Francis (1992).

It is rather surprising that Hilborn et al. present the analyses summarised in their fig. 2 and 3. In their first paragraph, they claim that the appropriate ("established") methodology for risk analysis is "Bayesian decision analysis" and that I should have either used this technique or shown that my procedure was superior to it. I will deal with this claim shortly, but first pose the question, "If Bayesian decision analysis is the appropriate methodology, why have Hilborn et al. not used it to demonstrate the alleged inadequacies of my method?"

It is true that the problem of risk estimation considered by Francis (1992) can be described in the framework of decision theory. But then, so can many standard problems of statistical inference (Ferguson 1967; Berger 1985). Are Hilborn et al. calling for a wholesale adoption of the decision theory paradigm in statistical analyses in fisheries science? Even were we to use a decision theoretic approach, it is not necessary to use a *Bayesian* analysis (Ferguson 1967; Berger 1985).

It is not clear whether Hilborn et al. realise that I have used "risk" in a sense different from that of decision theory. In my usage, risk is a probability (of something "bad" happening within a specific time period). This interpretation is widespread in the fisheries literature (Breiwick et al. 1981; Peterson and Smith 1982; Fletcher 1985; Brown and Patil 1986; Bergh and Butterworth 1987; Linder et al. 1987; Swartzman et al. 1987; Hall et al. 1988; Punt and Butterworth 1991; Restrepo et al. 1992), although not all authors explicitly define the term. (It is noteworthy that, in those of the cited papers where risk was actually estimated, Bayesian decision analysis was never used. Further, two of the four authors of Hilborn et al. are amongst the authors of the papers concerned.) In the decision theory framework, what I have called risk would be called the expected loss (where the expectation is over the "states of nature" space — in my case, over B_0).

The third paragraph of Hilborn et al. contains two puzzling statements. The opening statement — "Francis's method uses only the point estimate of the parameter B_0" — is patently wrong. The aim of the section on biomass estimation in my paper is to produce a probability distribution for B_0 (Francis 1992, fig. 1B). It is this distribution, rather than the point estimate of B_0, that is then used in estimating risk. The second statement — that my method "would assign alternative hypotheses about B_0 exactly the same probability under the two data sets [of their fig. 1]" — is not true of the method as described by Francis (1992), but is true of subsequent modifications of the method (Cordue and Francis 1992; Cordue et al. 1992). The issue at stake is what should be assumed about the

extent of variability of the biomass indices that I denoted O_i. Two approaches to this problem have been used. In one (Francis 1992), the variability is inferred (via a maximum likelihood procedure) from the degree of scatter in a plot like fig. 1 of Hilborn et al.; in the other (Cordue and Francis 1992; Cordue et al. 1992), this scatter is ignored and the variability is estimated (perhaps subjectively) from other evidence. I think it is a matter of judgement which approach is appropriate in any particular case. Moreover, I find the objection of Hilborn et al. to a consequence of the latter approach surprising, as they themselves have insisted on this approach (in a Bayesian setting) in their contributions to the New Zealand stock assessments of hoki (e.g., see the Bayesian section of Cordue et al. 1992).

Hilborn et al. ask what should be done when the cumulative distribution of Francis (1992, fig. 1A) does not reach 1. The answer is straightforward. In this case the probability distribution for B_0 (Francis 1992, fig. 1B) should be thought of as having a point mass at infinity. It may at first seem absurd to think of an infinite biomass having a nonzero probability. However, the point is that, in any particular application of the method, all "very large" values of B_0 are effectively the same (and effectively infinite) from the analyst's point of view. This is because they are so large that the given catch history has negligible effect on the biomass. In other words, the biomasses, B_i, are all (essentially) the same as B_0. (How large is "very large" will depend on the particular example; in the example of Francis (1992), a B_0 of 10^8 t is effectively infinite.) If the sequence of biomass indices, O_i, shows only a slight decrease (or an increase), there will be a nonnegligible probability, p, that they could have arisen from a situation in which $B_i = B_0$ for all i. Then the probability that B_0 is effectively infinite is p, and the asymptote for the cumulative distribution function should be $1 - p$.

Francis (1992) suggested that the value of scientific advice to fishery managers can be enhanced by expressing the uncertainty inherent in stock assessments in terms of biological risk. It would seem that Hilborn et al. do not disagree with this, but do object to the method that I have proposed for estimating risk. I have already said that mine is not the only way of estimating risk, and may not be the best (Francis 1992, p. 928, antepenultimate paragraph). However, I believe that the way to evaluate alternative estimators is to see how well they perform *as estimators of risk*. Their behaviour in estimating B_0 is of secondary importance. Readers wishing to see such an evaluation are referred to Cordue and Francis (1992), in which the confidence distribution estimator of Francis (1992) is compared with Bayesian, bootstrap, and maximum likelihood estimators — R.I.C.C. Francis, *Fisheries Research Centre, Ministry of Agriculture and Fisheries, Box 297, Wellington, New Zealand.*
(JB799)

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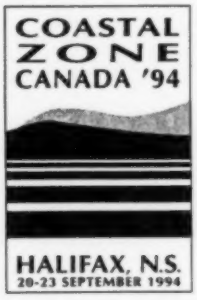
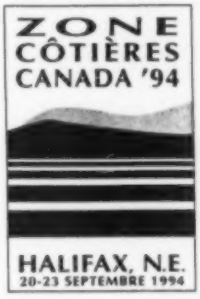


Coastal Zone Canada '94
(CZC'94)

World Trade and Convention Centre,
Halifax, Nova Scotia
20–23 September 1994

Zone Côtière Canada '94
(ZCC'94)

World Trade and Convention Centre,
Halifax (Nouvelle-Écosse)
20–23 septembre 1994



The theme of this major international conference is "Cooperation in the Coastal Zone," and its purpose is to provide participants from many different disciplines and interest groups opportunities to explore strategies for developing new partnerships in coastal zone management. Cooperative approaches and new working relationships among coastal zone stakeholders will be examined in formal papers, posters, panel sessions, workshops, and round-table discussions. A major Trade Show and Exhibition is also planned to allow companies and organizations to meet with other conference attendees.

CZC'94 will be broad in scope and will involve, among others, scientists and engineers, academics, government officials, primary resource users, industry and business, the military, aboriginal groups, and special-interest organizations.

Papers are invited in the following areas (other coastal and ocean-related subjects will be considered, as will proposals for case-study or round-table sessions):

Environmental Science and Engineering

Remote sensing; wetlands; sedimentation modelling; baseline studies and monitoring; dunes and coastal vegetation; shoreline stabilization; pollution control and research; coastal processes; habitat conservation; storms and weather monitoring; coastal mapping and GIS; environmental remediation; new technologies.

Resource Management

Oil and gas; fisheries and aquaculture; dumping and dredging; oil spill management; living resource conservation; ocean mining; sand and gravel; aboriginal rights and resource management; energy.

Community Initiatives and Coastal Development

Empowerment of coastal communities; public access; education and community initiatives; marine and coastal parks and sanctuaries; tourism and recreation; access to information/scientific knowledge; socioeconomic issues; communities affected by change.

Cette grande conférence internationale a pour thème « la coopération dans la zone côtière » et pour but de donner l'occasion aux participants de diverses disciplines et de divers groupes d'intérêt d'explorer des stratégies de former de nouveaux partenariats en gestion de la zone côtière. Des méthodes de collaboration et de nouvelles relations de travail entre les intervenants de la zone côtière seront examinées dans des communications formelles, des séances d'affichage, des tribunes, des ateliers et des tables rondes. Une foire et une exposition commerciales d'envergure sont aussi prévues pour permettre aux sociétés et aux organismes de recontrer les autres participants à la conférence.

ZCC'94 sera d'une grande portée et rassemblera notamment des scientifiques et des ingénieurs, des universitaires, des fonctionnaires, des utilisateurs de ressources primaires, des industriels et des commerçants, des militaires, des groupes autochtones et des groupes d'intérêt particuliers.

Les participants sont invité à présenter des mémoires dans les domaines suivants (d'autres sujets liés aux zones côtières et marines seront examinés, ainsi que des propositions d'études de cas ou de tables rondes) :

Science et génie de l'environnement

Télédétection; terres humides; modélisation de la sédimentation; études et surveillance de base; végétation des dunes et des littoraux; stabilisation des rivages; dépollution et recherche; processus côtiers; conservation des habitats; tempêtes et surveillance météorologique; cartographie des côtes et SIG; remise en état de l'environnement; nouvelles technologies.

Gestion des ressources

Pétrole et gaz; pêches et aquiculture; décharge et dragage; gestion des déversements de pétrole; conservation des ressources vivantes; exploitation minière sous-marine; sable et gravier; droits des autochtones et gestion des ressources; énergie.

Initiatives communautaires et développement côtier

Délégation de pouvoirs aux communautés côtières; accès public; éducation et initiatives communataires; parcs et

Coastal Jurisdictional Issues

Waterfront and harbour planning; land use planning; interagency planning and coordination; maritime security; legal issues; conflict resolution.

Regional and International Issues

Bilateral arrangements; comparative management studies; local versus regional approaches; technology transfer; governmental responsibilities and structures; regional waterbody management; global environmental change; island states.

The deadline for submission of abstracts is **September 30, 1993**, and for submission of manuscripts is **March 31, 1994**.

Conference Co-chairs are Mr. Larry Hildebrand, Environment Canada Protection and Conservation, and Mr. Brian Nicholls, Marine Assessment and Liaison, Bedford Institute of Oceanography (P.O. Box 1006, Dartmouth, N.S., Canada B2Y 4A2). Secretariat phone: (902) 429-9497; Fax (902) 429-9491.

sanctuaires marins et côtiers; tourisme et loisirs; accès à l'information/connaissance scientifique; questions socioéconomiques; communautés touchées par le changement.

Questions de droit côtier

Planification des rivages et des ports; planification des utilisations des terres; planification et coordination entre les organismes; sécurité maritime; questions légales; résolution de conflits.

Questions régionales et internationales

Ententes bilatérales; études comparatives de gestion; approche locale ou régionale; transfert de technologie; responsabilités et structures des administrations publiques; gestion régionale des plans d'eau; changement environnemental planétaire; états insulaires.

La date limite est le **30 septembre 1993** pour la présentation des résumés et le **31 mars 1994** pour la présentation des manuscrits.

Les co-présidents de la conférence sont : M. Larry Hildebrand, Protection et conservation, Environnement Canada, et M. Brian Nicholls, Évaluation et liaison marines, Institut océanographique de Bedford (B.P. 1006, Dartmouth (N.-É.), Canada B2Y 4A2). Téléphone secrétariat : (902) 429-9497; télécopieur : (902) 429-9491.

International Conference on Naturally Produced Organohalogens

14–17 September 1993, Delft, The Netherlands

Natural production of organohalogens has been known for a long time, and in a few cases it has been shown that natural processes can result in substantial contributions of specific low-molecular-weight compounds. Only recently, however, has the full extent of the natural production of organohalogens begun to be revealed. Measurements of group parameters, such as adsorbable organohalogens (AOX), have shown that organohalogens are more widespread than previously assumed. The quantities detected often exceed the amount that can be explained by human activities in surrounding areas. An extensive, nonspecific halogenation of organic matter has been shown to occur in terrestrial environments; photochemical formation of reactive chlorine species in the atmosphere is another potential source of organohalogens.

Although many organohalogens are pollutants (exclusively of anthropogenic origin), the existence of a sharp border with contaminants (both anthropogenic and natural) can be questioned. Natural halogenation processes, in which reactive chlorine is formed as an intermediate, have obvious similarities to industrial bleaching and disinfection processes. In addition, anthropogenic emissions could interact with natural halogenation processes in several ways. The aim of the conference is to bring together specialists in environmental pollution, biohalogenation, and abiotic formation of reactive halogens to assess the extent and the environmental significance of the natural production of organohalogens.

English will be the working language of the conference. The conference will be held at the TNO-complex Zuidpolder, Schoemakerstraat 97, Delft, The Netherlands.

Conférence internationale sur les halogènes organiques produits dans la nature

14–17 septembre 1993, Delft, Pays-Bas

La production naturelle d'halogènes organiques est un phénomène connu depuis longtemps, et il a été démontré que, dans certains cas, ce processus naturel peut produire un grand nombre de composés spécifiques à faible poids moléculaire. Toutefois, ce n'est que récemment que l'on a saisi toute l'ampleur de la production d'halogènes organiques. Des mesures basées sur des paramètres applicables à des séries chimiques, comme les halogènes organiques adsorbables (HOA), ont révélé que les halogènes organiques étaient beaucoup plus répandus qu'on ne le supposait. Les quantités relevées dépassaient souvent le seuil attribuable à l'activité humaine dans la région visée. On a démontré qu'une forte halogénation non spécifique se produisait dans les milieux terrestres; en outre, la formation par processus photochimique d'espèces de chlores réactifs dans l'atmosphère est une autre source possible d'halogènes organiques.

Bien que de nombreux halogènes organiques soient des substances polluantes (d'origine exclusivement anthropique), on peut mettre en doute la nette distinction établie entre celles-ci et les contaminants (d'origine à la fois anthropique et naturelle). Les processus naturels d'halogénation, au cours desquels la formation de chlore réactif constitue une étape intermédiaire, présentent certaines similarités évidentes avec les processus industriels de blanchiment ou de décontamination. Par ailleurs, les émissions anthropiques pourraient influer de plusieurs façons sur les procédés d'halogénation naturelle. L'objectif visé par les organisateurs de la conférence consiste à réunir des spécialistes de la pollution de l'environnement, de la biohalogénation et de la formation non biotique d'halogènes réactifs afin

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13th International Diatom Symposium

1–7 September 1994
Hotel Villa del Mare
Acquafredda di Maratea (Potenza), Italy

The 13th Symposium of the International Society for Diatom Research will be organized in Italy by the Marine Botany and the Benthic Ecology Laboratories of the Stazione Zoologica "Anton Dohrn" (Naples). It will be held in Acquafredda di Maratea, an attractive fishing village located about 200 km south of Naples; the venue is easily reached by train or coach from both Naples and Rome.

The Symposium will cover all aspects of diatom research: morphology, cytology, genetics, reproductive biology, physiology, biochemistry, molecular biology, ecology, biogeography, paleontology, phylogeny, and systematics. Oral presentations and posters are solicited on marine as well as freshwater diatoms, living as well as fossil. The deadline for preregistration is **31 October 1993** and for receipt of abstracts is **31 March 1994**. The proceedings of the Symposium will be published by Biopress, Bristol, U.K.

The first notice has been sent to members of the Society and other diatomists known to the conveners of the Symposium, but anyone who has not received this can obtain details of lecture topics, accommodation, costs, etc., from the Conference Secretariat: Jean Gilder Congressi snc, 13th International Diatom Symposium, via G. Quagliariello 35/E, I-80131 Napoli, Italy; telephone: +39(0)81 546 3779 or 545 4617; facsimile: +39(0)81 546 3781. The conveners of the meeting are D. Marino, M. Montresor, L. Mazzella, and M.C. Buia, Stazione Zoologica "Anton Dohrn", Villa Comunale, I-80121 Napoli, Italy.

d'évaluer l'ampleur et l'incidence environnementale de la production d'halogènes organiques dans la nature.

La conférence, dont la langue de travail sera l'anglais, sera tenue au complexe TNO Zuidpolde, Schoemakerstraat 97, Delft, Pays-Bas.

Pour de plus amples renseignements, écrire à l'adresse suivante : Congress Office ASD, P.O. Box 40, 2600 AA Delft, The Netherlands. Téléphone : +31 15 120234; télécopieur : +31 15 120250.

13^{e} Symposium international sur les diatomées

1–7 septembre 1994
Hôtel Villa del Mare
Acquafredda di Maratea (Potenza), Italie

Le 13^{e} Symposium de la Société internationale de recherche sur les diatomées sera organisé en Italie par les Laboratoires de botanique marine et d'écologie benthique de la Stazione Zoologica « Anton Dohrn » (Naples). Il aura lieu à Acquafredda di Maratea, un joli village de pêche situé à environ 200 km au sud de Naples; il est facile de s'y rendre par train ou par autobus à partir de Naples ou de Rome.

Le Symposium portera sur tous les aspects de la recherche sur les diatomées : morphologie, cytologie, génétique, biologie de la reproduction, physiologie, biochimie, biologie moléculaire, écologie, biogéographie, paléontologie, phylogénie et systématique. Des présentations orales et des séances d'affichage sur les diatomées tant marines que d'eau douce, vivantes et fossiles, sont sollicitées. La date limite de préinscription est le **31 octobre 1993** et la date limite de réception des résumés est le **31 mars 1994**. Les actes du Symposium seront publiés par Biopress, Bristol, Royaume-Uni.

Le premier avis a été envoyé aux membres de la Société et à d'autres diatomistes connus des organisateurs du Symposium, mais quiconque n'a pas été rejoint peut obtenir des détails sur les sujets des présentations, l'hébergement, les frais, etc., en s'adressant au Secrétariat de la conférence : Jean Gilder Congressi snc, 13^{e} Symposium international sur les diatomées, via G. Quagliariello 35/E, I-80131 Napoli, Italie; téléphone : +39(0)81 546 3779 ou 545 4617; télécopieur : +39(0)81 546 3781. Les organisateurs de la conférence sont D. Marino, M. Montresor, L. Mazzella et M.C. Buia, Stazione Zoologica « Anton Dohrn », Villa Comunale, I-80121 Napoli, Italie.

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211 plates

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Description du développment de la morue d'éclosion jusqu'à 54 jours, d'après des spécimens examinés au microscope optique, au microscope à transmission et au microscope électronique à balayage et d'après des montages d'éléments squelettiques entiers. La déscription des stades ultérieurs, jusqu'à la métamorphose, est basée sur des micrographies de morues sauvages prises au microscope optique ; ces morues provenaient d'échantillons de plancton recueillis par chalutage.

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